Abbreviated Identification of Bacteria and Yeast

NCCLS Guideline M 35 A

Mary K. York, Ph.D. ABMM

References Prove Value

- Doern, G., R. Vautour, M. Gaudet, and B. Levy. 1994. Clinical impact of rapid *in vitro* susceptibility testing and bacterial identification.
  - MIC 9.6 h from colonies vs. 25.9 h- showed less mortality, length of stay and orders of laboratory tests
- Barenfanger, J., C. Drake, and G. Kacich. 1999. Clinical and financial benefits of rapid bacterial identification and antimicrobial susceptibility testing.
  - evaluated evening vs. next day - 5 h difference - length of stay and cost was significantly less

Pros and Cons of Rapid Methods

- Pros
  - Less work than standard methods
  - Results are out faster
  - Less cost
- Cons
  - Requires technical expertise for accuracy
  - Cannot be applied to all cases
  - May disrupt workflow

Criteria Used by Committee for Rapid Tests

- Only for specific organisms
- Errors must not have a negative impact on patient care
- Accuracy must be > 95%
- Emphasis on organisms that have unique reactions
- Results are not presumptive if all criteria met-cpt 4 code issue

Factors to Keep in Mind

- Not for direct specimens
- All conditions must be met
- Keep isolate for future testing if needed

Technologist must….

- Begin with pure colony
- Recognize what it could be from typical colony morphology
- Perform rapid tests accurately and read them correctly
- Often do Gram stain

Supervisor must…..

- Validate the competency of the staff doing tests
- Check to see that all tests are done
- Be sure that procedures are written and QC is done at appropriate intervals

CLIA ‘88

- *All quality control activities must be documented.*
- *The laboratory must check positive and negative reactivity with control organisms*
- *Each new lot/shipment of reagents, commercial tests, or biochemical test media prior to being used on patient specimens.*

CLIA ‘88

➔ *and*

(1) Each day of use for catalase, coagulase, beta-lactamase, and oxidase reagents and DNA probes;

(2) Each week of use for Gram stain, bacitracin, optochin, ONPG, X and V discs or strips; and

(3) Each month of use for antisera…

Does not address ID disks, rapid indole, Staph Latex reagents, etc…...

CLIA 2003

- *All quality control activities must be documented*
  - *The laboratory must check positive and negative reactivity with control organisms*
    - *(1) Each day of use for DNA probes and beta-lactamase (ex. cefinase);*
    - *(2) Each week of use for Gram stain and AFB stains; and*
    - *(3) Every 6 months of use for antisera…*
    - *(4) Each new lot/shipment of reagents, commercial tests, or biochemical test media prior to being used on patient specimens.*

Organisms covered by NCCLS

Gram Negative Bacilli

- ***Escherichia coli***
- ***Haemophilus influenza***
- ***Moraxella catarrhalis***
- ***Proteus mirabilis/penneri***
- ***Proteus vulgaris***
- ***Pseudomonas aeruginosa***

Gram Positive Cocci

- ***Enterococcus* species**
- ***Staphylococcus aureus***
- ***Streptococcus agalactiae***
- ***Streptococcus pneumoniae***
- ***Streptococcus pyogenes***

Yeast

- ***Candida albicans***
- ***Candida glabrata***
- ***Cryptococcus neoformans***

Organisms covered by NCCLS

Anaerobic Gram Negative Bacilli

- ***Bacteroides fragilis* group**
- ***Bacteroides urealyticus***
- ***Bilophila wadsworthii***
- ***Prevotella* species**
- ***Prevotella intermedia***
- ***Porphyromonas* species**
- ***Fusobacterium nucleatum***

Anaerobic Gram Negative Cocci

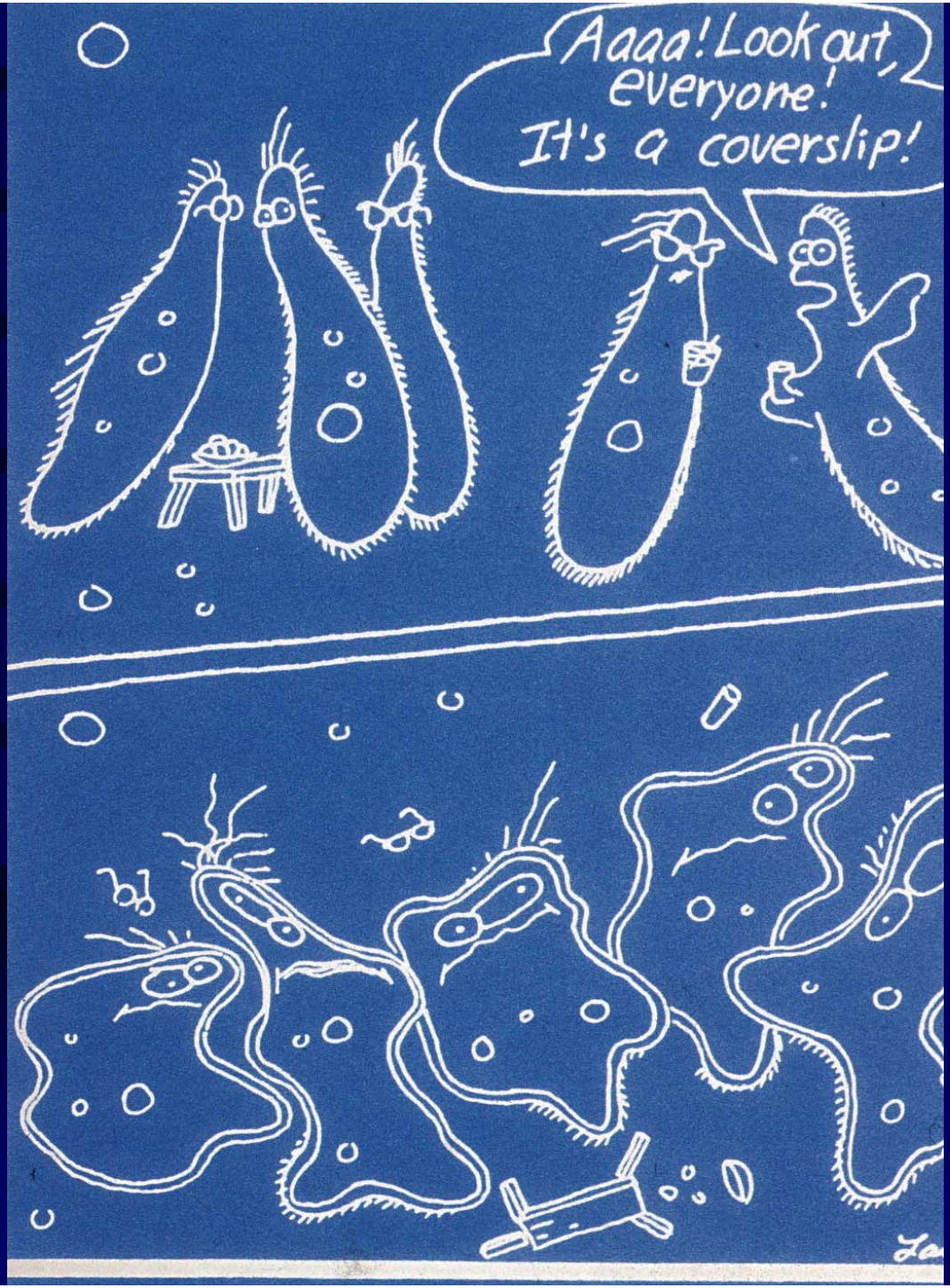
- ***Veillonella* species**

Anaerobic Gram Positive Bacilli

- ***Clostridium difficile***
- ***Clostridium perfringens***
- ***Clostridium septicum***
- ***Clostridium sordellii***
- ***Clostridium tetani***
- ***Propionibacterium acnes***

Anaerobic Gram Positive Cocci

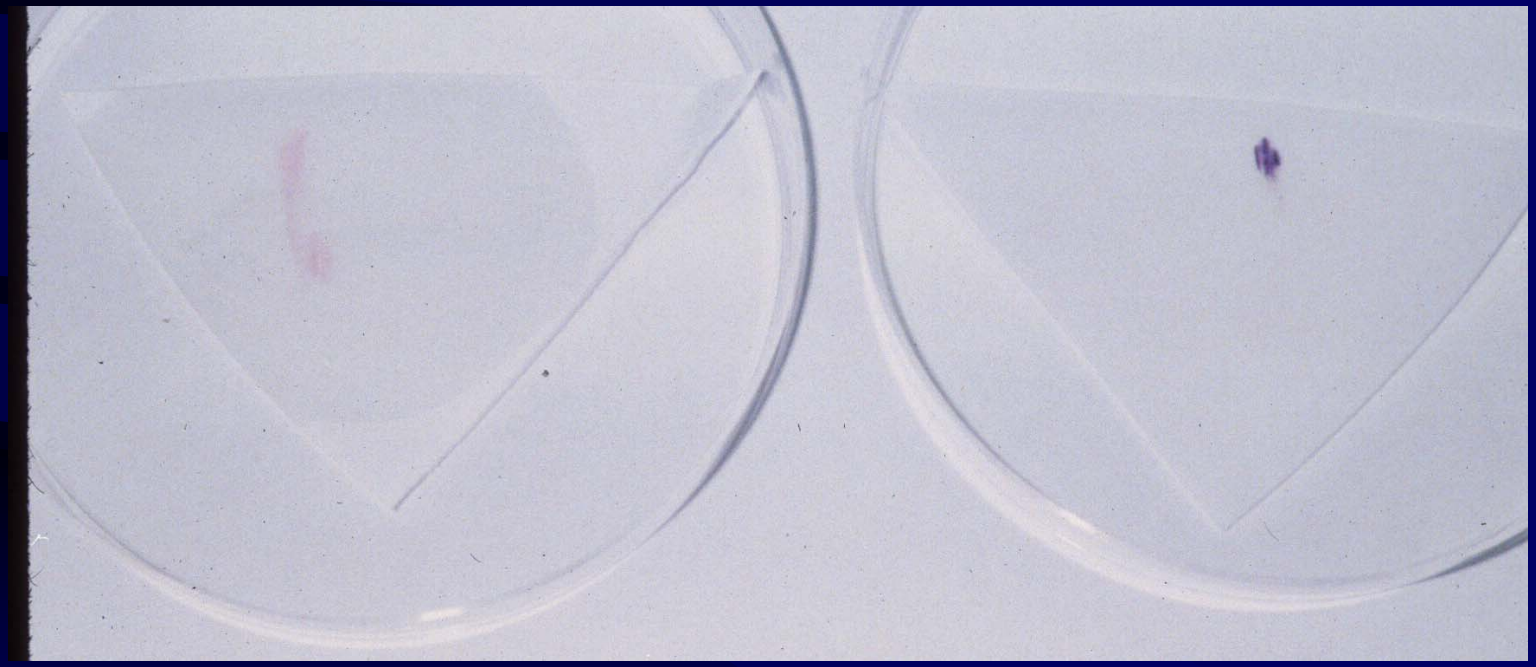
- ***Peptostreptococcus* species**


Aaaa! Look out, everyone! It's a coverslip!

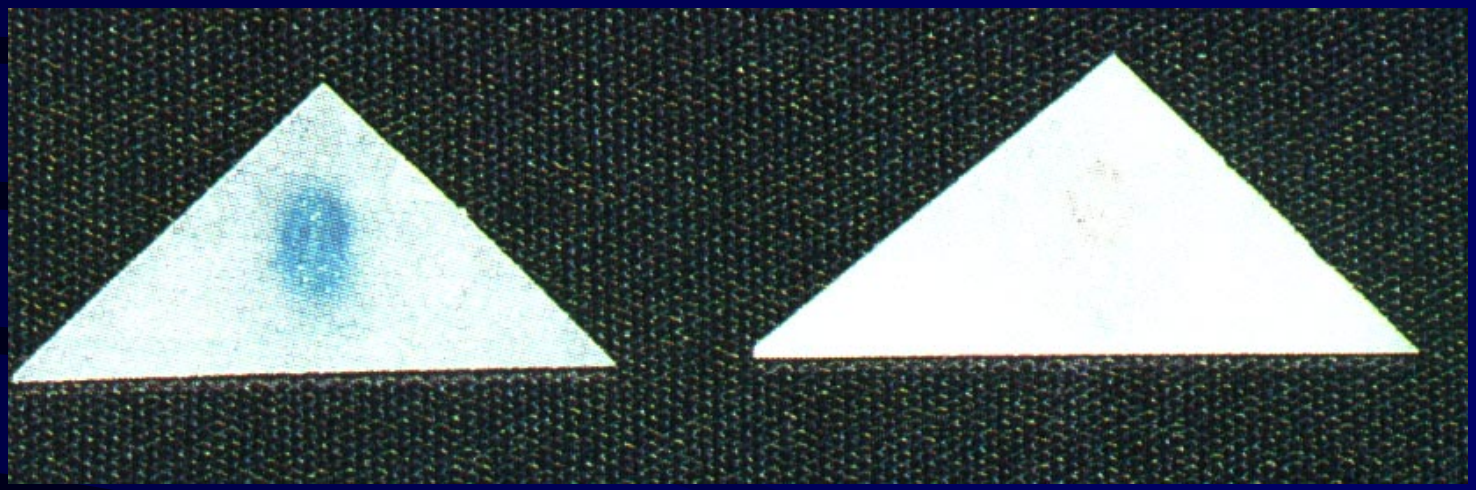
Start with indole and oxidase

Indole

Oxidase

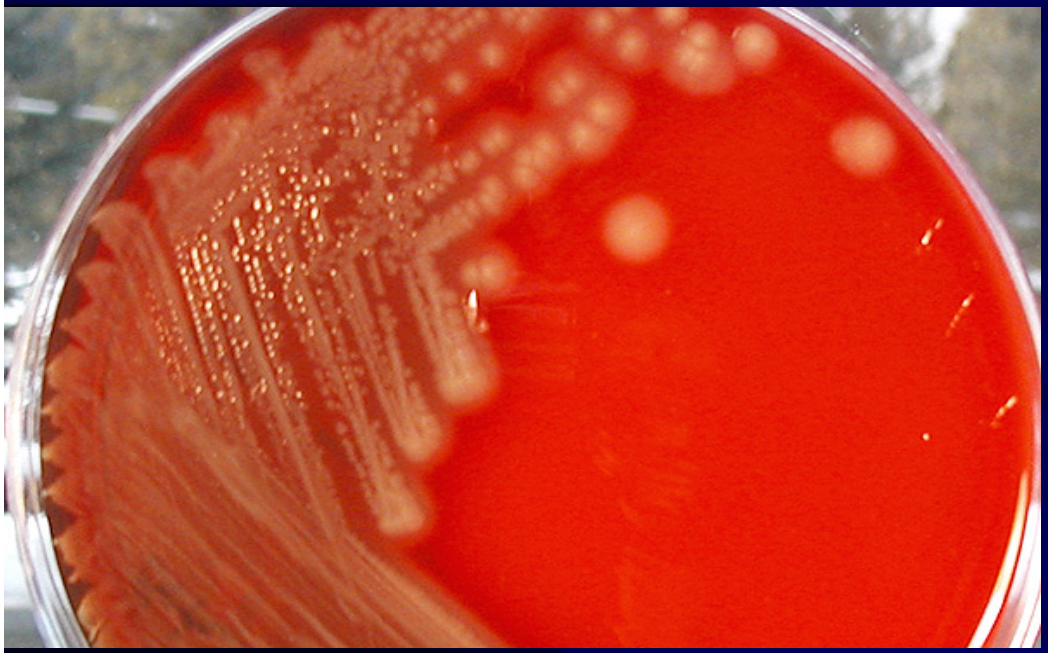


Cinnamaldehyde reagent



Not as immediate
Disappears faster
Is the reagent required for anaerobic spot indole

Identification of *E.coli*



- Indole +
- Oxidase -
- Gram-negative rod
- Beta-hemolytic

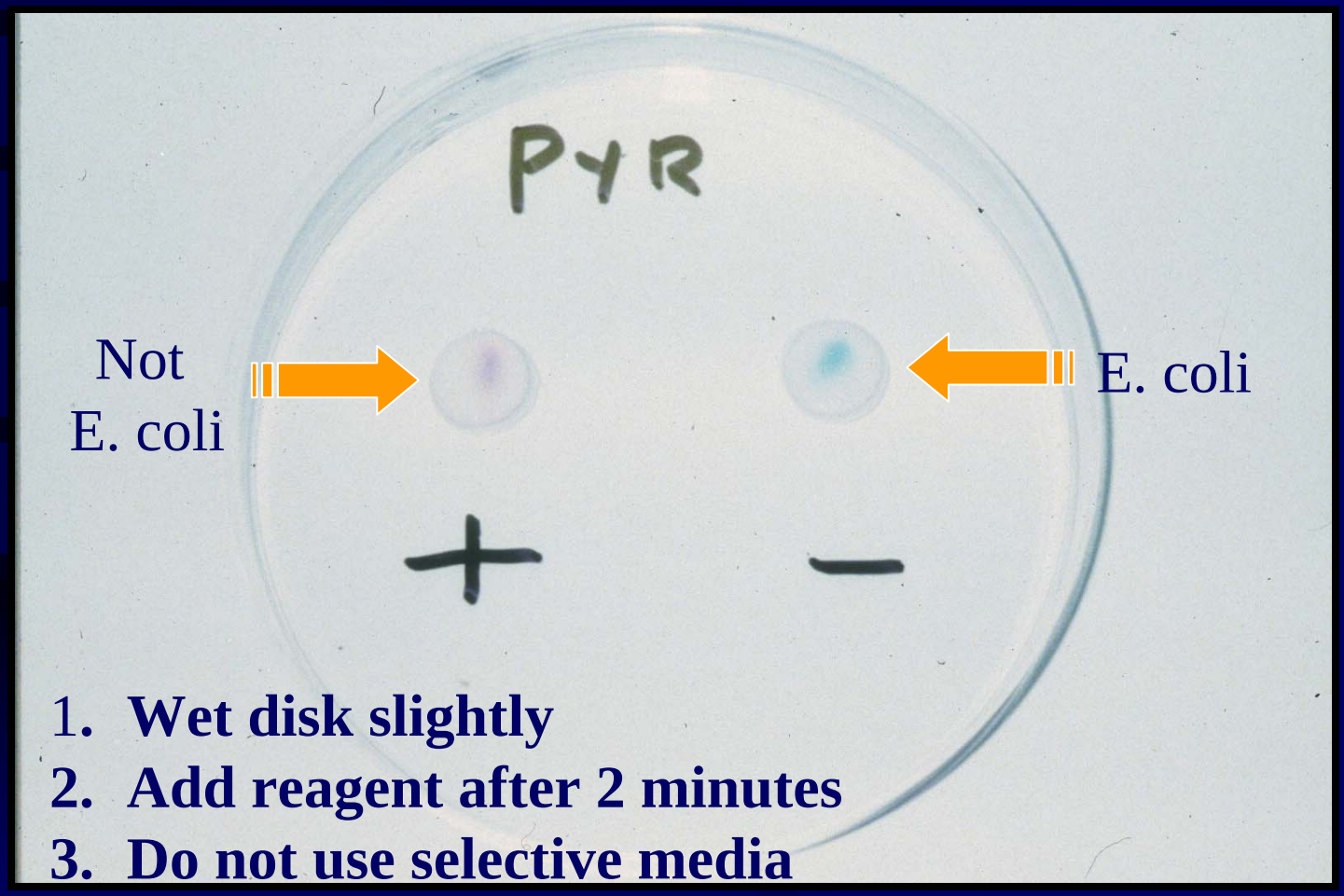
= *E. coli*

Limitation: some *Proteus* and *Morganella* and all *Edwardsiella* are hemolytic. These species are lactose-negative.

What if it is not hemolytic?

Lactose + and ….

PYR negative = *E. coli*

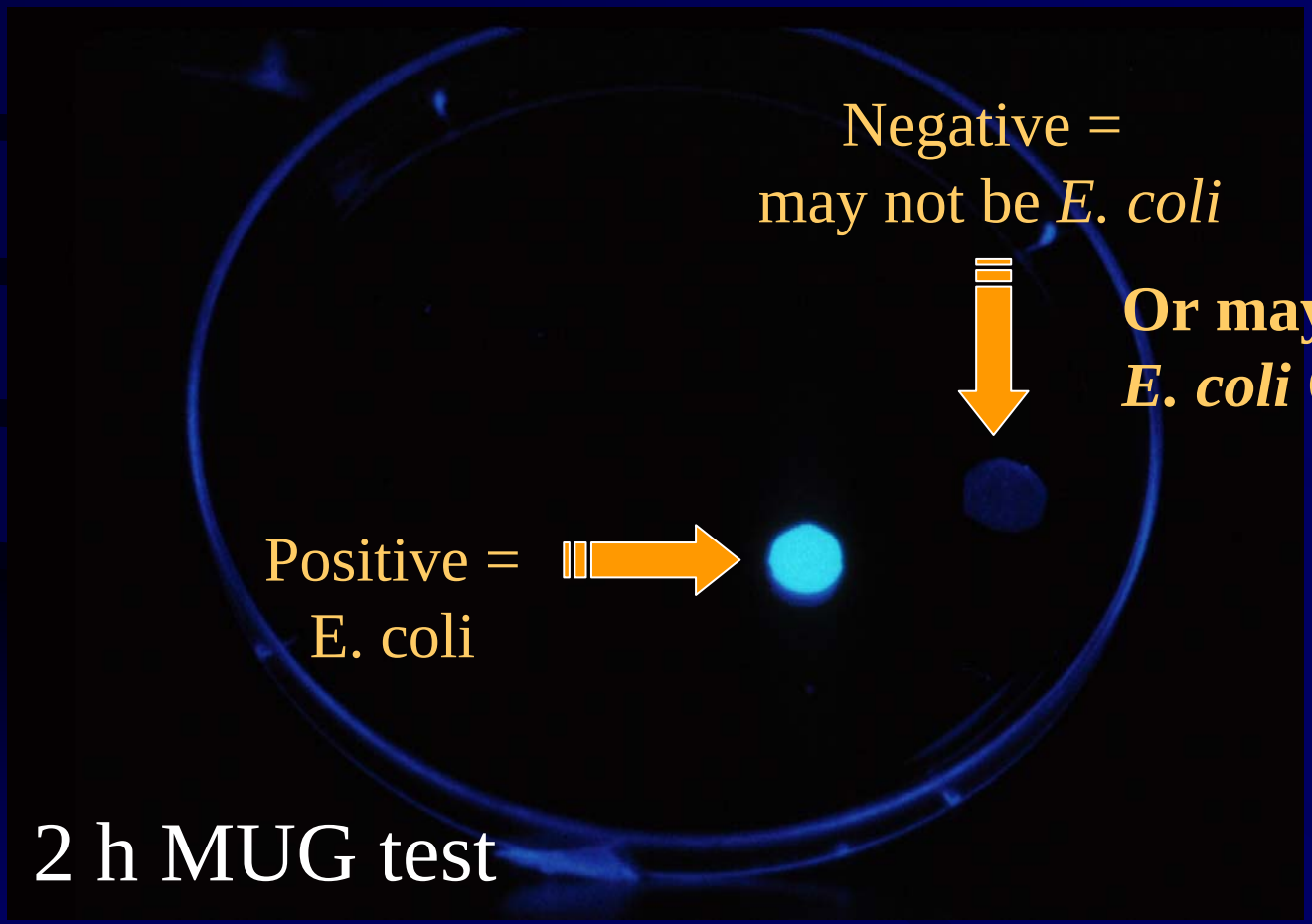


1. **Wet disk slightly**
2. **Add reagent after 2 minutes**
3. **Do not use selective media**

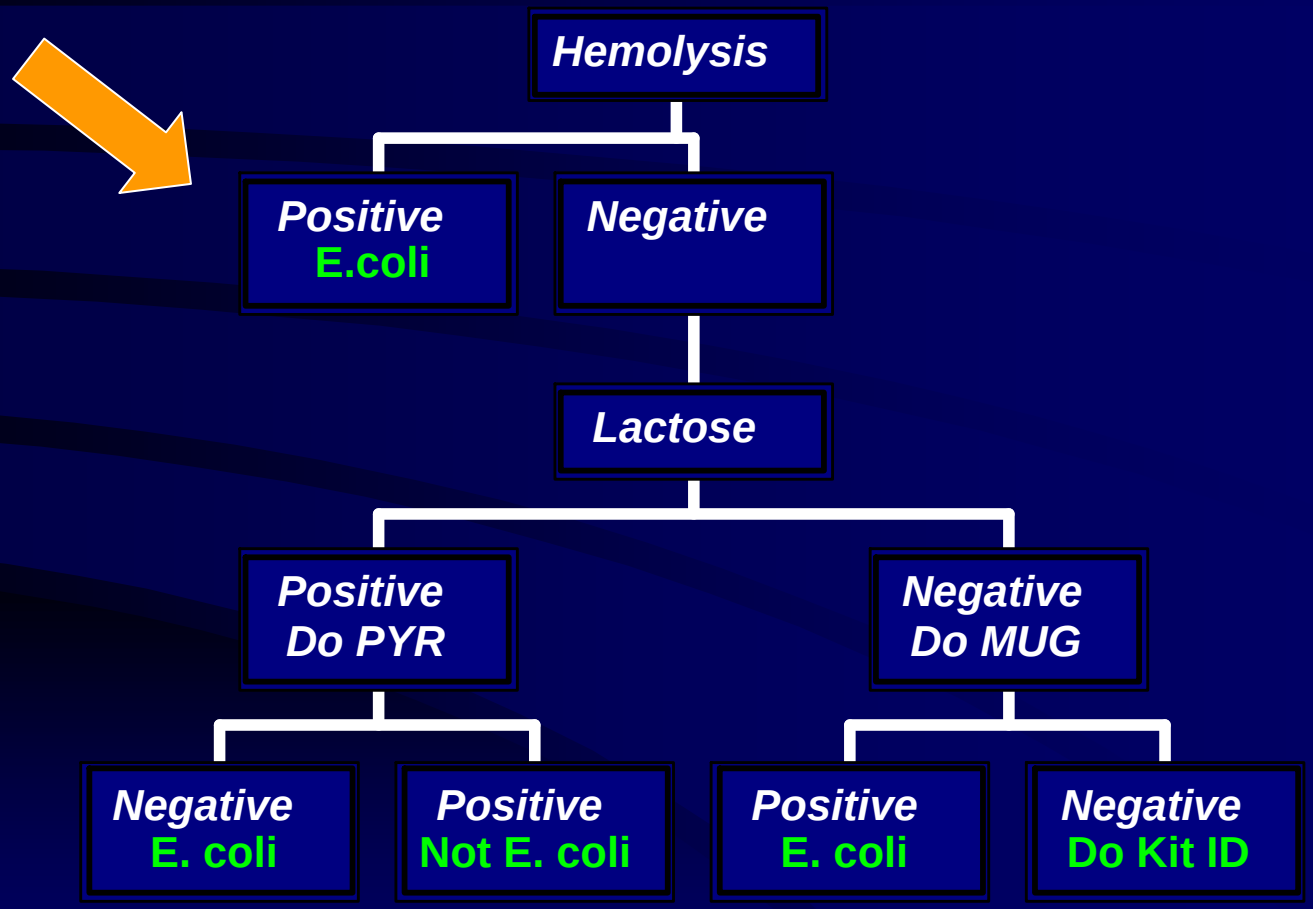
PYR reactions of GNR

Genus	Indole	Lactose	PYR
Citrobacter	*Some +*	*V*	+
E. coli	+	*V*	-
Klebsiella	*Some +*	+	+
Yersinia	*Some +*	-$^{+}$	+
Enterobacter	-	+$^{-}$	+
Serratia	-	+$^{-}$	+
Morganella	+	-	-
Proteus	*Some +*	-	-
Providencia	+	-	-
Edwardsiella (beta hemolytic)	+	-	-
Shigella	*Some +*	-	-
Salmonella	-	-$^{+}$	-

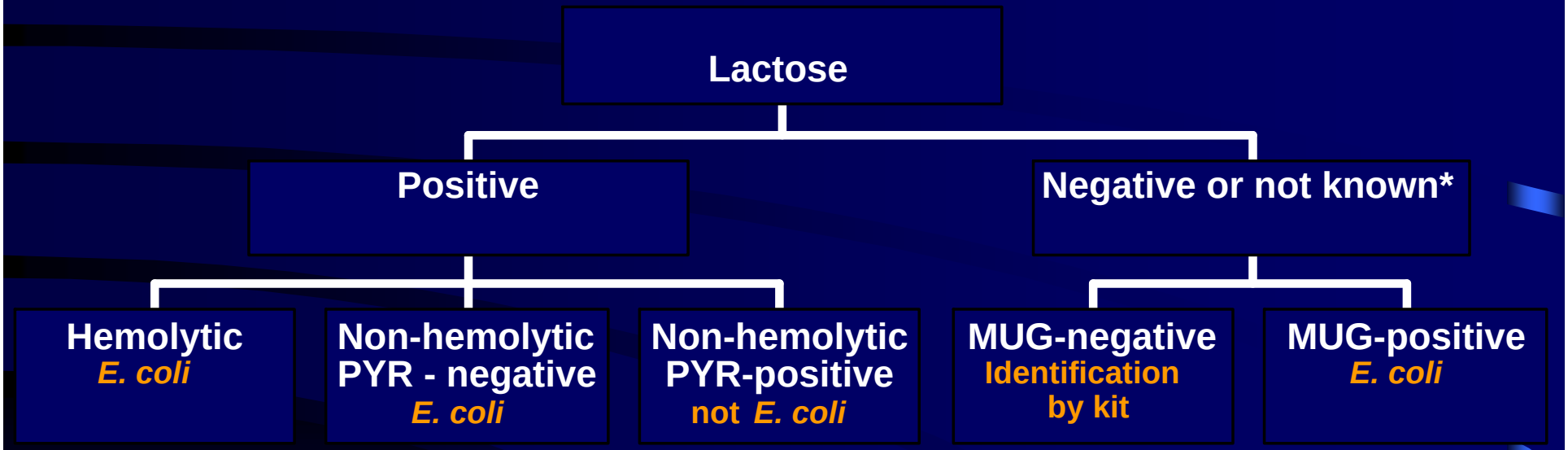
What if it is not lactose-positive?



CLSI Algorithm for E. coli: Indole +; Oxidase –



Algorithm for some labs... Indole + and oxidase -



Limitations

- Beta hemolytic, lactose-negative should be limited to UTI and geographic areas that lack hemolytic *Morganella* and *Proteus*.
- Take colony from BAP that corresponds to MAC or EMB
- Do not use MUG for GI specimens, except to identify *E. coli* O157.

Accuracy of 1064 Indole + oxidase- strains = 99.7%

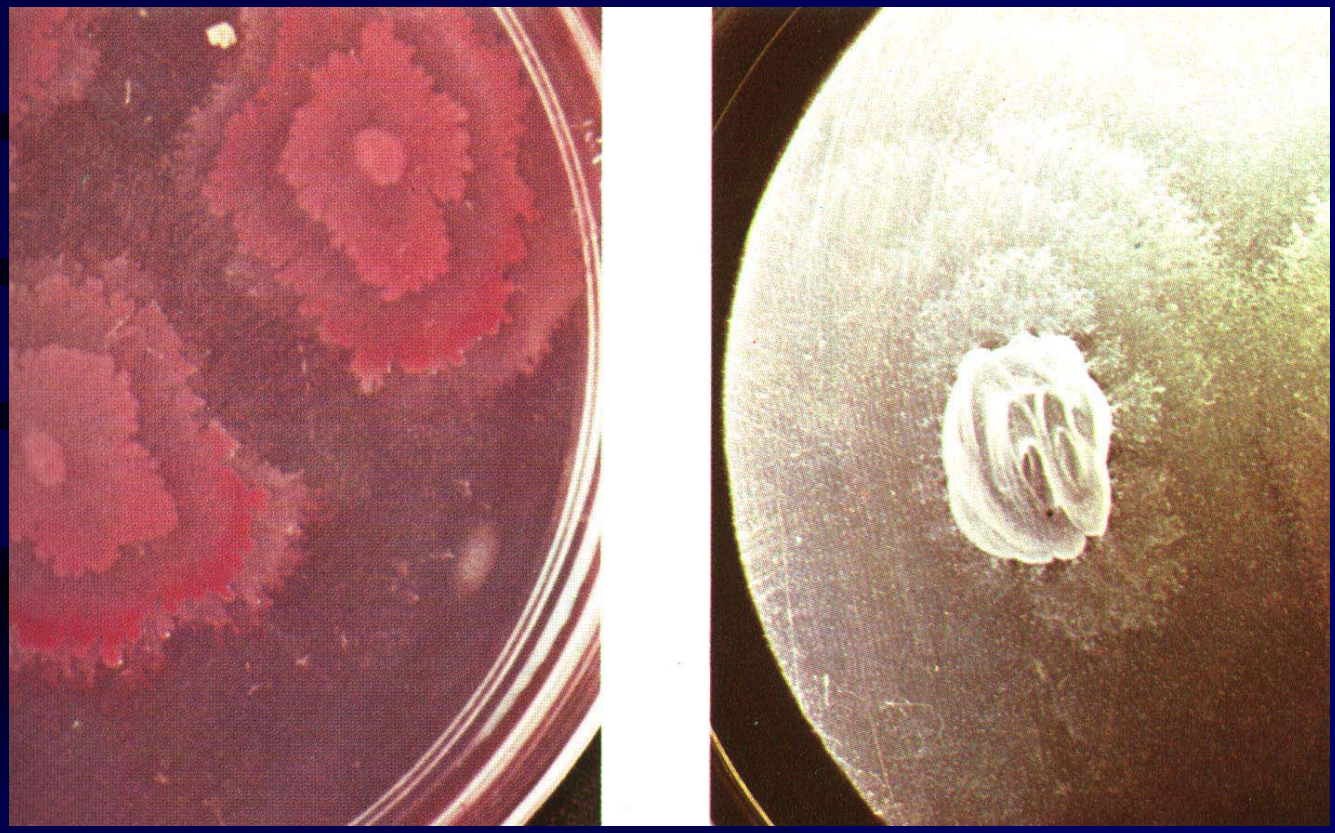
- 294 were hemolytic and *E. coli*.
- 628 were lactose positive and PYR negative and *E. coli*.
- 65 were MUG positive and *E. coli*.
- 13 were MUG negative and needed kit to identify as *E. coli*.
- 64 were not *E. coli* but 3 were called *E. coli*

Cost savings

- $3100 in reagents - 987 kits omitted
- 70 hours of technologist time

York, M.K., E.J. Baron, M. Weinstein, R Thomson, and J.E. Clarridge. 2000. A Multi-Laboratory validation of rapid spot tests for identification of *Escherichia coli*. J Clin Microbiol 38: 3394-3398.

Spreading *Proteus*



MacConkey

BAP

Proteus identification= Spreading colony plus….

- Indole-positive: → ***Proteus vulgaris***
- Indole-negative: → Ampicillin-susceptible: ***Proteus mirabilis***

  → Ampicillin-resistant

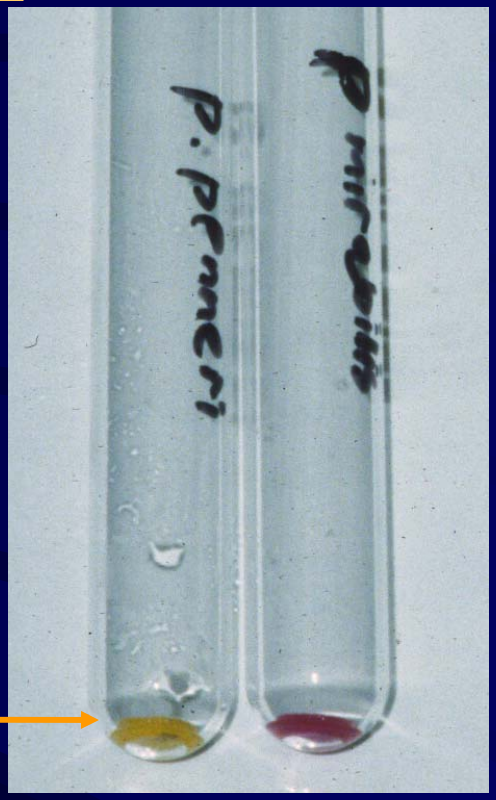
  → Maltose-negative or Ornithine-positive: ***P. mirabilis***

  → Maltose-positive or Ornithine-negative: ***P. penneri***

Proteus species

	Spreading on BAP	Indole	Ampicillin S	ODC	Maltose ferment
P. mirabilis	**96**	**2**	**95**	**99**	**0**
P. vulgaris	**64**	**98**	**0**	**0**	**97**
P. penneri	**65-90**	**0**	**0**	**0**	**100**

Rapid maltose



Positive

Rapid Urea

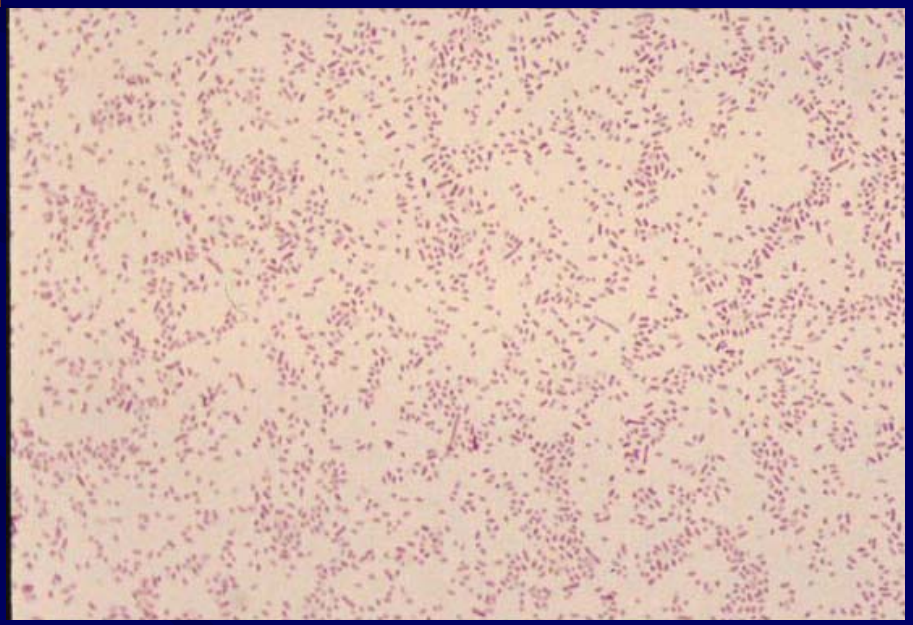
- Make suspension of organism
- Add disk
- Incubate 2 h
- Read urea
- Add HCl -not nec.
- Add $FeCl_3$

Other Rapid Urea Positive species

- *Brucella*
- *Helicobacter pylori*
- *Bordetella* species
- Some *Corynebacterium*

- Grows on BAP
- Coccobacillus
- Catalase -positive
- Oxidase - positive
- Urease - positive

- This is *Brucella* unless proven otherwise
- Work in a BSC
- Confirm with serology
- Reportable disease
- Bioterrorism

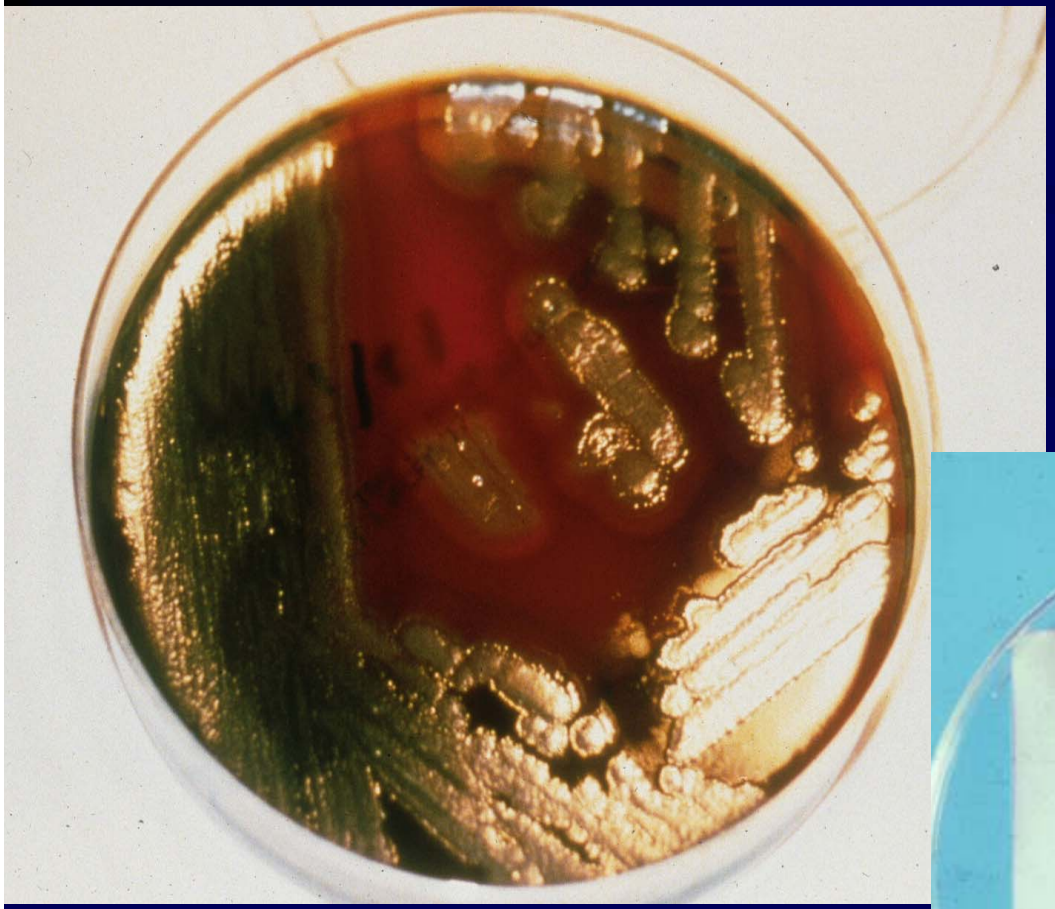


Brucella Diagnosis

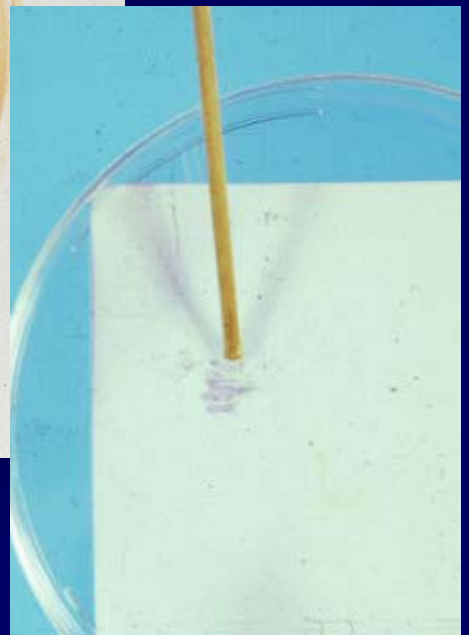
- Symptoms are non-specific
- Onset is insidious
- Risks are eating raw dairy products or working in a microbiology laboratory
- Without diagnosis there is no appropriate treatment:

  Doxycycline plus rifampin

Pseudomonas aeruginosa



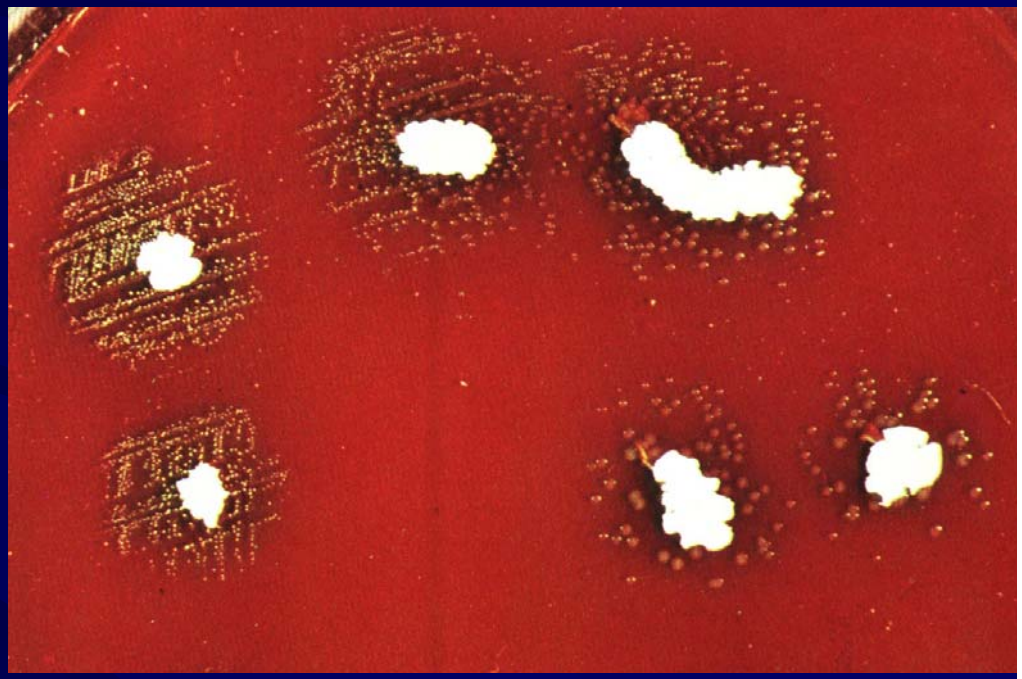
- **Oxidase +**
- **Indole -**
- **Metalic or mucoid**
- **Fruity odor**



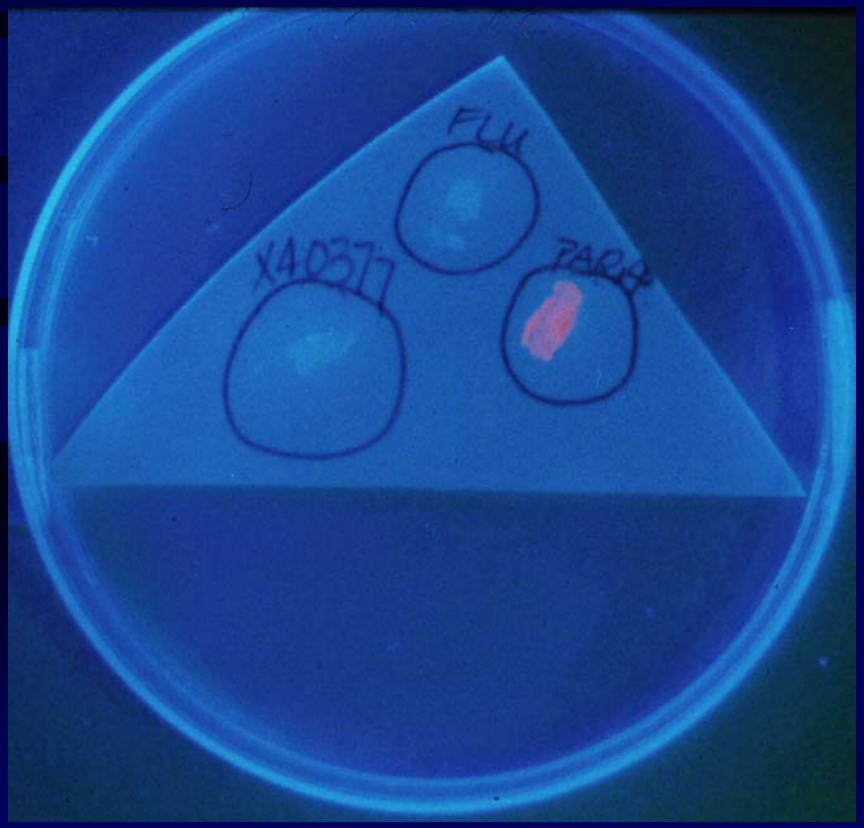
CF patients: Confirm with colistin or poly B disk

Identification of *Haemophilus influenzae*

- Small gram-negative coccobacilli
- Growth of large colonies only on CHOC in 24 h or around staphylococci
- And…..



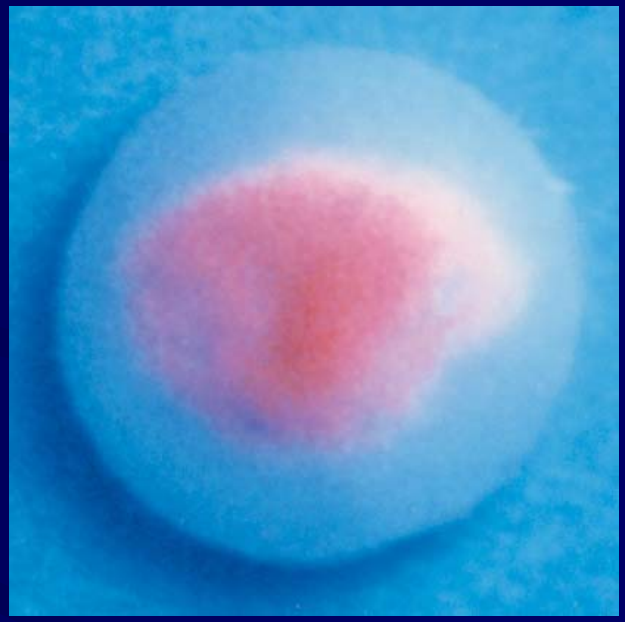
ALA test negative - 2 h 35°C read under UV light



1. ***Francisella*** **grows on CHOC in 48 h but is small colony**
2. ***Cannot differentiate H. influenzae*** **from *H. haemolyticus;* the latter is hemolytic on horse or rabbit blood agar.**

- You can either make the reagent or buy it

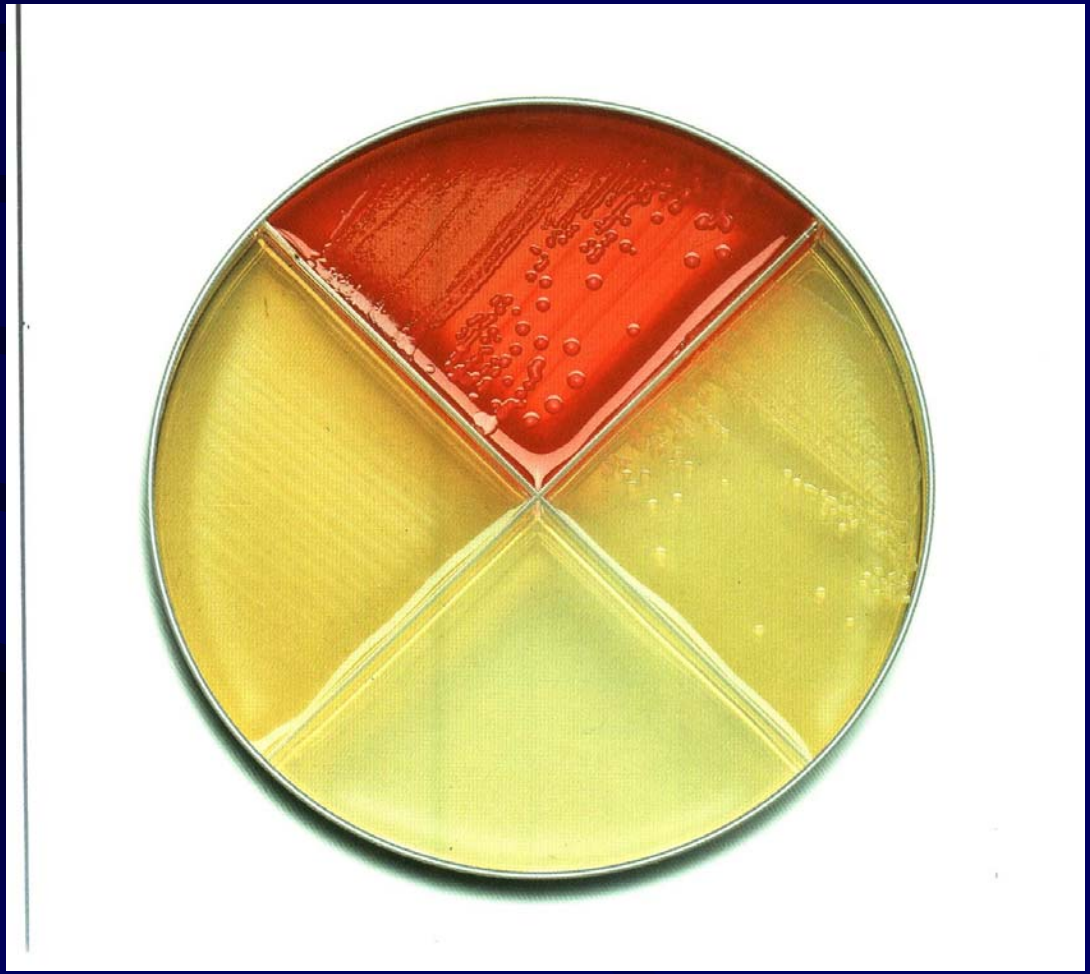
- Recipe in NCCLS M35-A and handout



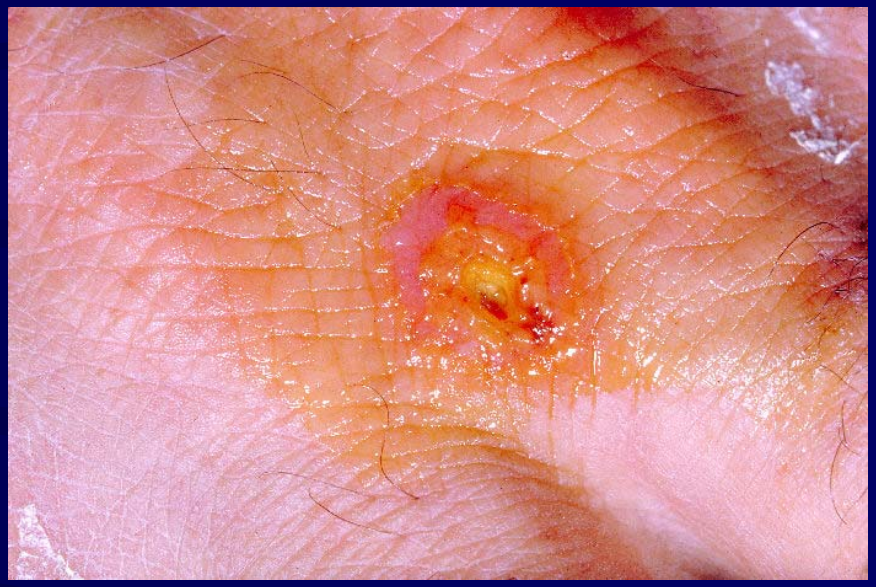
Haemophilus species

	SATELLITE V factor	ALA	LACT	UREA	IND	ODC	CAT
H. influenzae	+	-	-	V	V	V	+
H. haemolyticus	+	-	-	+	V	-	+
H. parahaemolyticus	+	+	-	+	-	-	+
H. parainfluenzae	+	+	-	V	V	V	V
H. paraphrophilus	+	+	+	-	-	-	-
H. ducreyi	-	-	-	-	-	-	-

Horse Blood Agar



Case Study



- 36 y/o male with HIV
- Camped in Yosemite
- Non-healing, erythematous 3 mm cyst on neck
- FNA aspirate and biopsy
- Gram stain negative
- GNR grew on chocolate in 3 days
- Patient treated with ciprofloxacin & did well

Day 3 - GNR growing on Choc

catalase +; urease -; oxidase -

Vitek NHI = 99% Actinobacillus actinomycetemcomitans

Day 4 - Satellite-negative

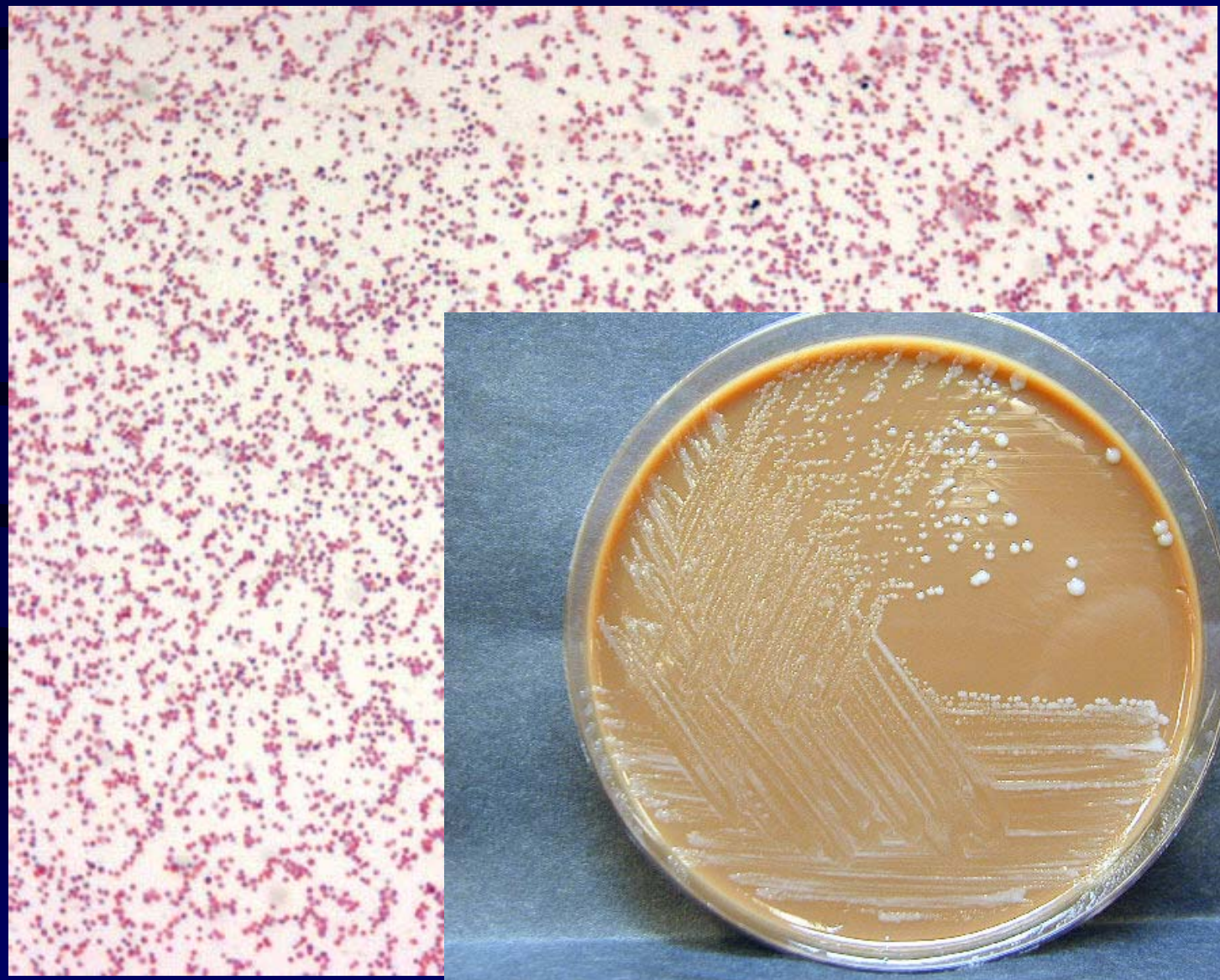
ALA neg; motility -; NH 2520 No ID

Day 5 - Tech sets up MIC - no growth day 7

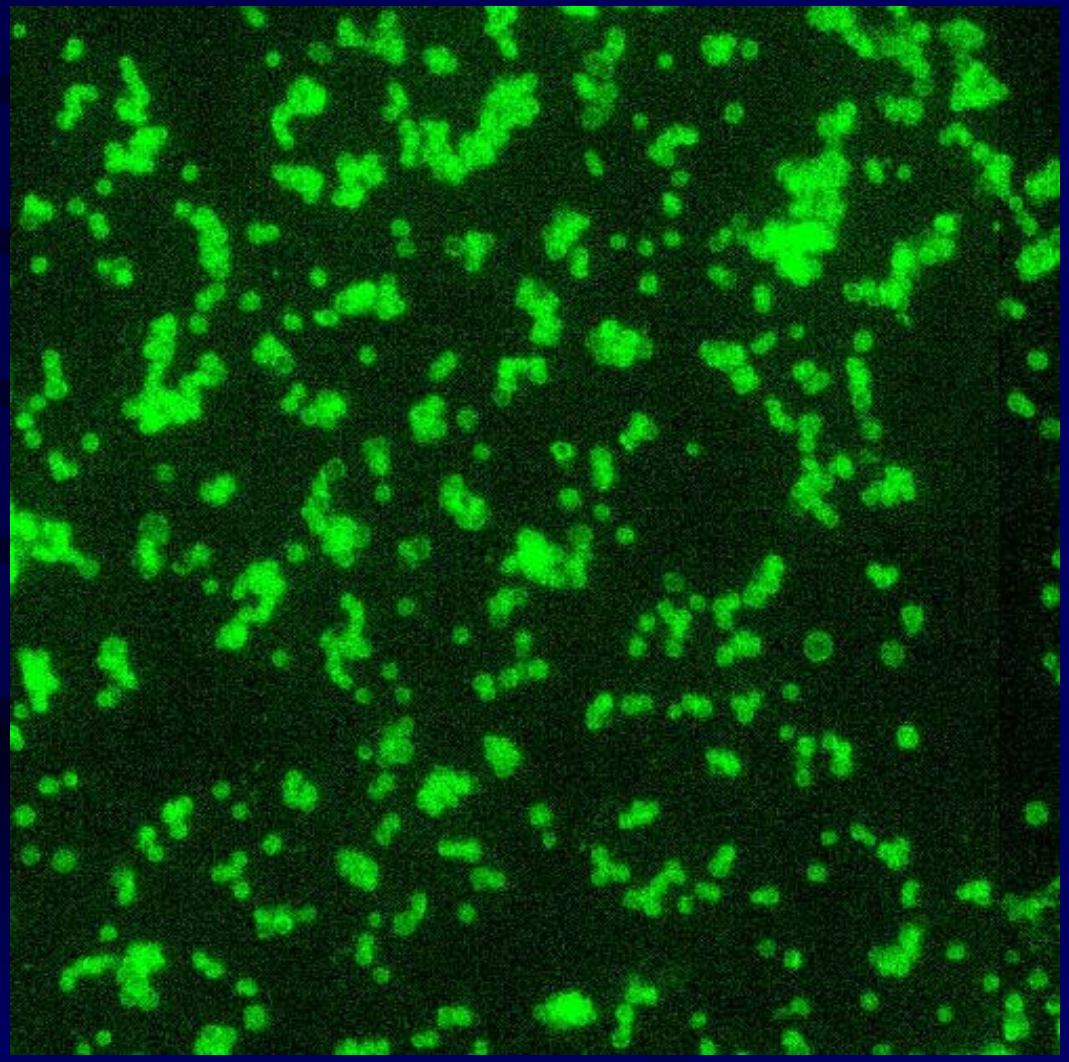
Day 10 - KB set up

Day 12 - KB penicillin R

Day 40 - MDL reports ID-done by FA

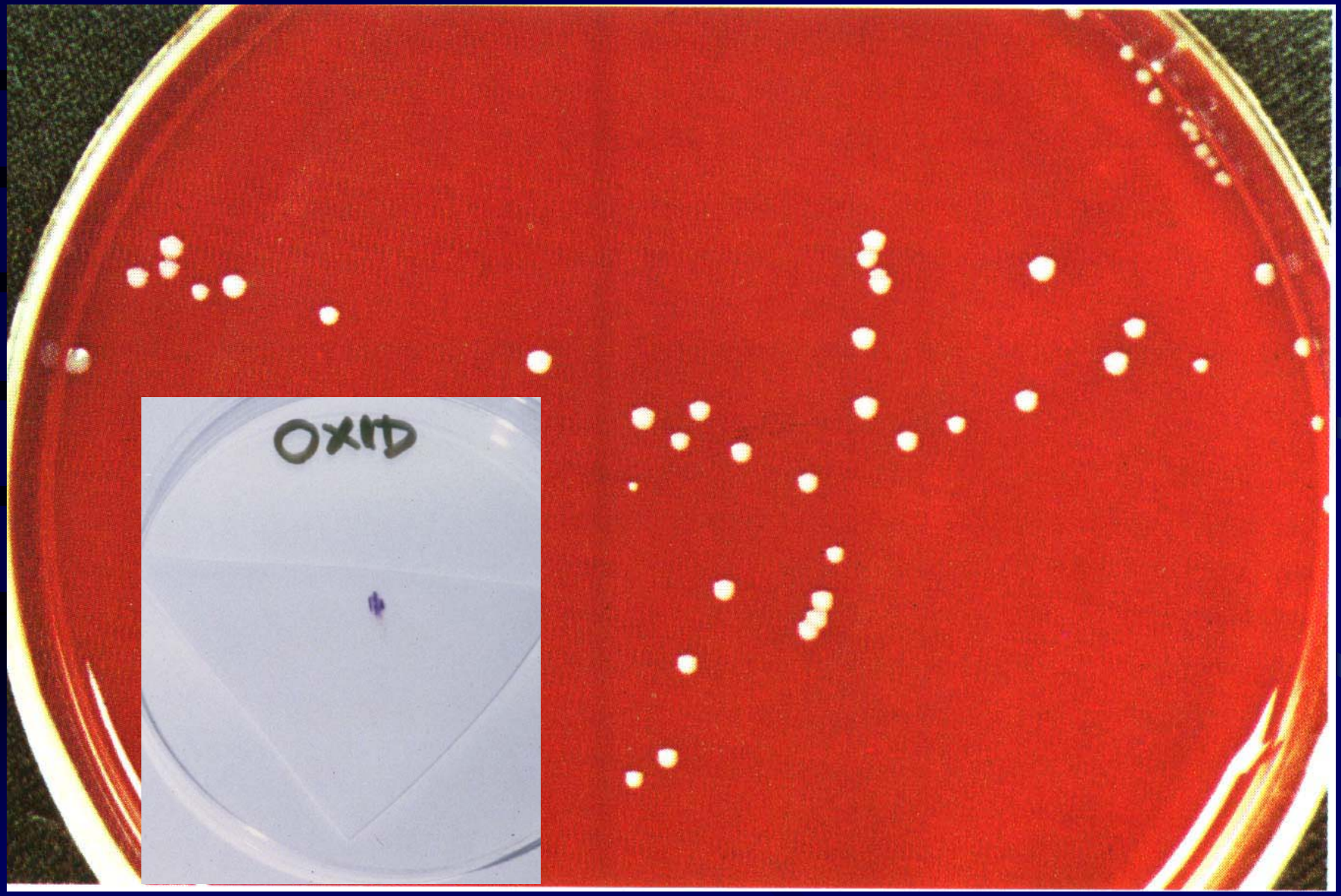


Francisella tularensis - BSL III

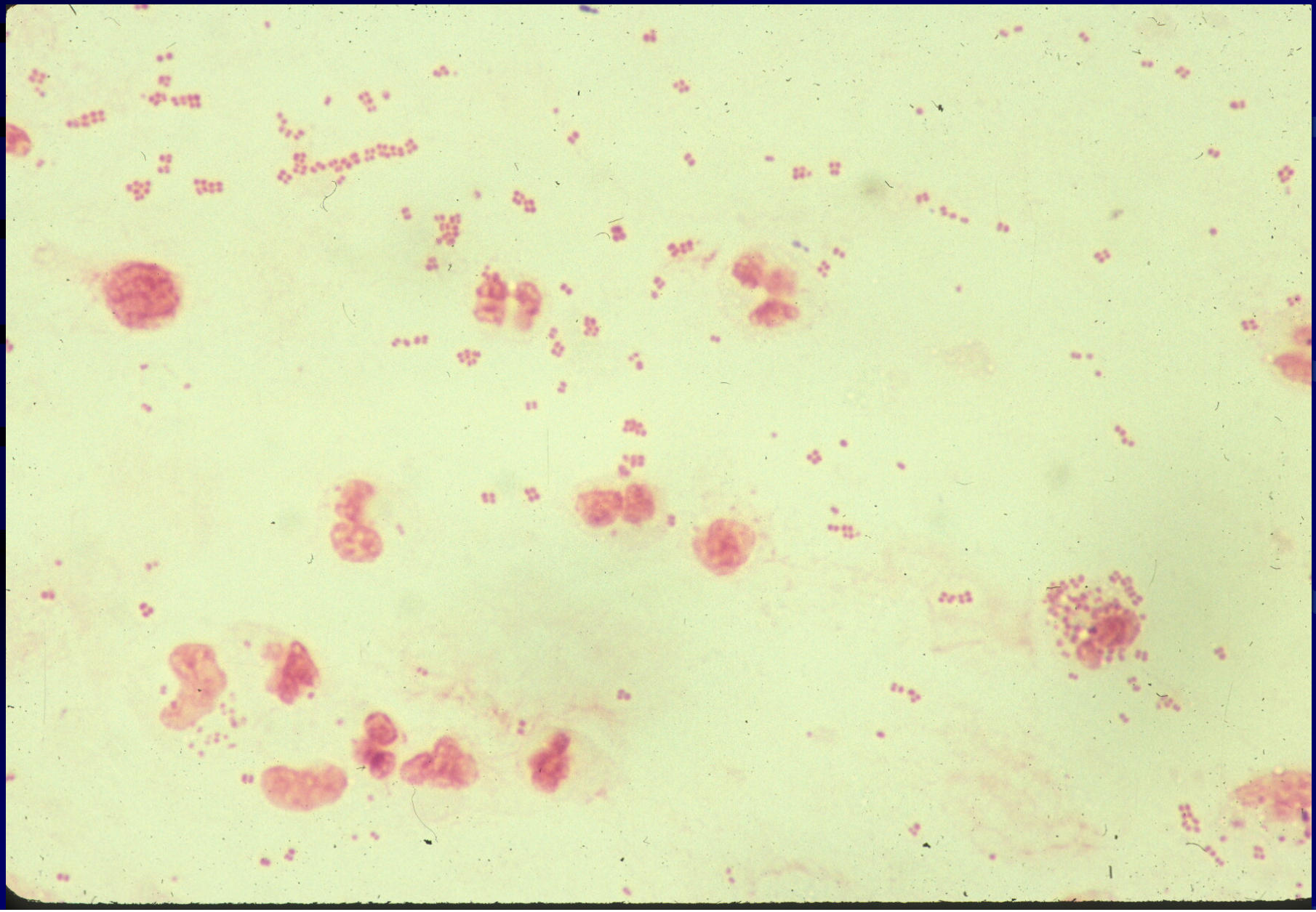


Francisella tularensis

- If growth takes longer than 24 h, may be *Francisella* which will not satellite,
  - ALA-negative.
  - Grows on CHOC but not on BAP.
  - Oxidase-negative; catalase weak.
  - No kit will identify
  - Do cefinase-result is positive.
  - Send to health department if cefinase-positive
- Rabbit fever, tick, mosquito & fly bites
- Bioterrorism

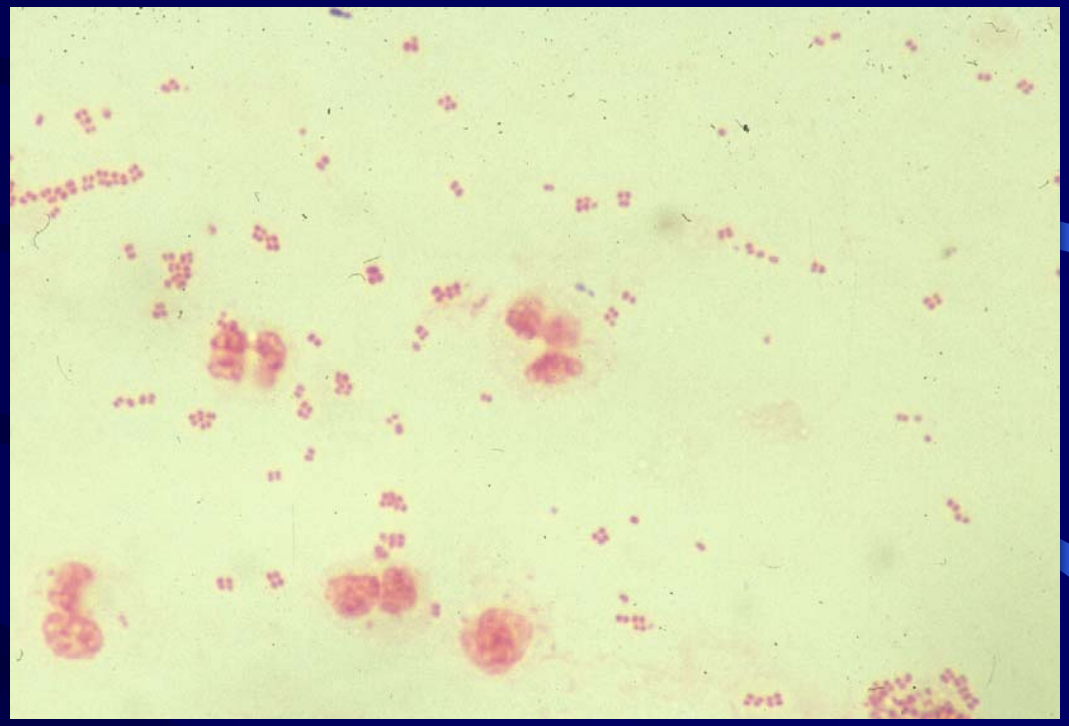

OXID

Moraxella catarrhalis



Confirmatory Identification of *M. catarrhalis*

- Gram negative diplococci
- Grows on BAP
- Oxidase-positive
- Butyrate (or indoxyl acetate) positive in 5 min



Actually all *Moraxella* are butyrate + but the others are coccobacilli

- Inoculate plate and put Pen disk on it
- Perform Gram stain from around the disk
- Do on subs of positive blood cultures - polymyxin B and vancomycin too!

- May use indoxyl acetate
- Same method and color
- Can be used for identification of *Campylobacter jejuni*

  (C. lari is negative*)*

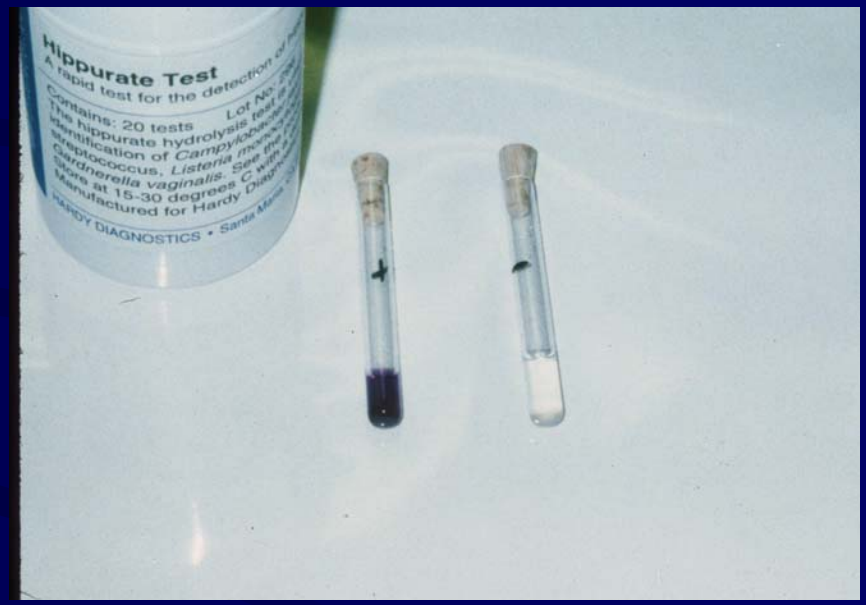
Campylobacter jejuni

- Requires microaerobic environment
- Oxidase - positive
- Catalase - positive
- Curved rod
  - Hippurate - positive
  - If hippurate - negative; indoxyl acetate -positive and cefazolin R identifies
  - Nal Acid or cipro R & cefazolin R & indoxyl acetate -negative is

    C. lari

Rapid Hippurate

- Inoculate broth
- Incubate 2 h
- Add ninhydrin
- Observe blue color



In Summary for Gram negative rods

- If growing on MAC, do
  - Indole
  - Oxidase
  - Kit if not *E. coli*, *Proteus* or *Pseudomonas*
- If not growing on MAC, do
  - Catalase
  - Oxidase
  - Gram stain
  - Generally kits are not helpful here

Staphylococcus aureus

Tube coag

Slide coag

Latex agglutination

Identification of *S. aureus*

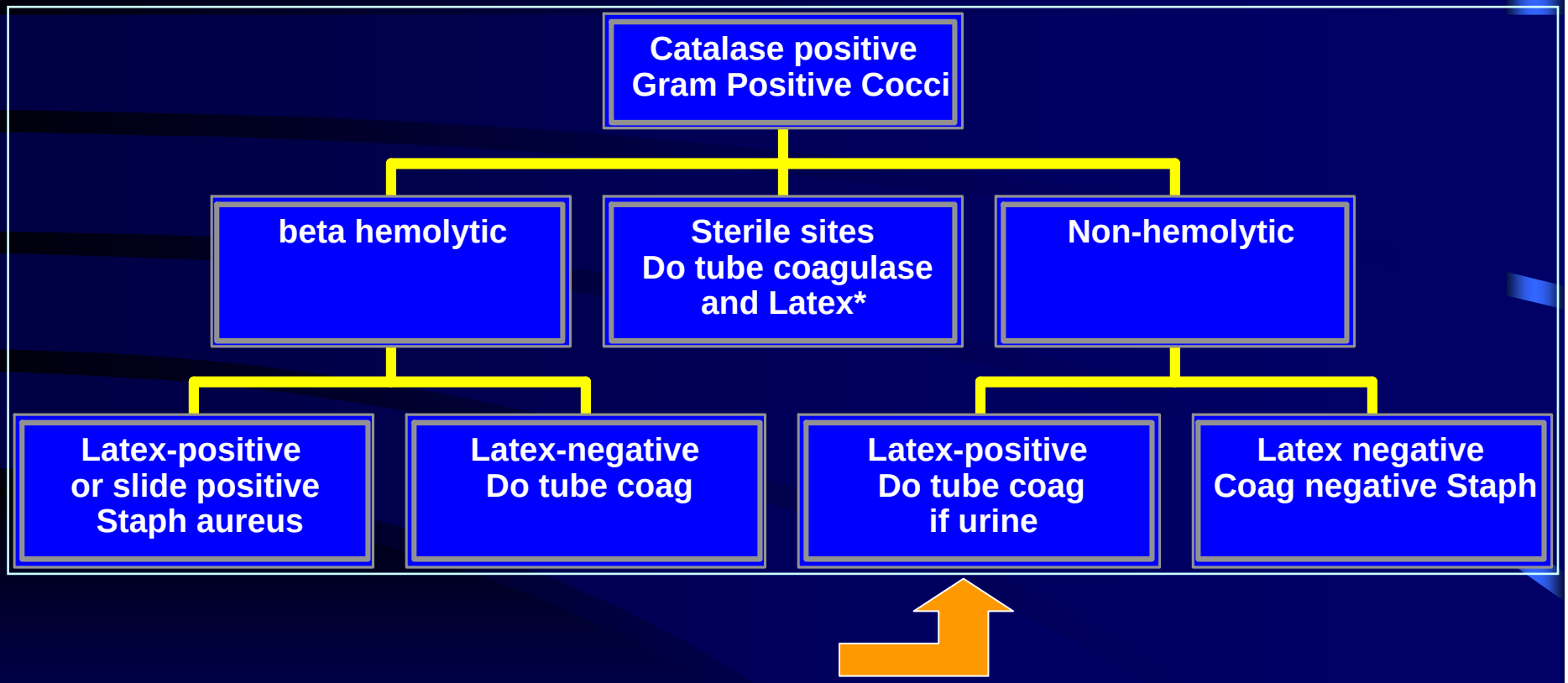
- Grows as opaque, white colony
- May be beta hemolytic
- Catalase-positive
- Gram-positive cocci in clusters
- Slide, tube or agglutination positive

Rapid tube coagulase is 4 h at 35°C; then must go to 25°C for 20 h more if negative

Limitations

- Rapid Tube Coag is 4 h at 35°C; then must go to 25°C for 20 h more if negative
- S. *lugdunensis* and *S. schleiferi* can be slide and Latex positive
- *S. saprophyticus* (and rare others) can be Latex positive
- MRSA can be Latex negative

Algorithm for Latex testing for Staphylococci



Hemolysis is defined as present in 18 h - not 48 h; not under colony

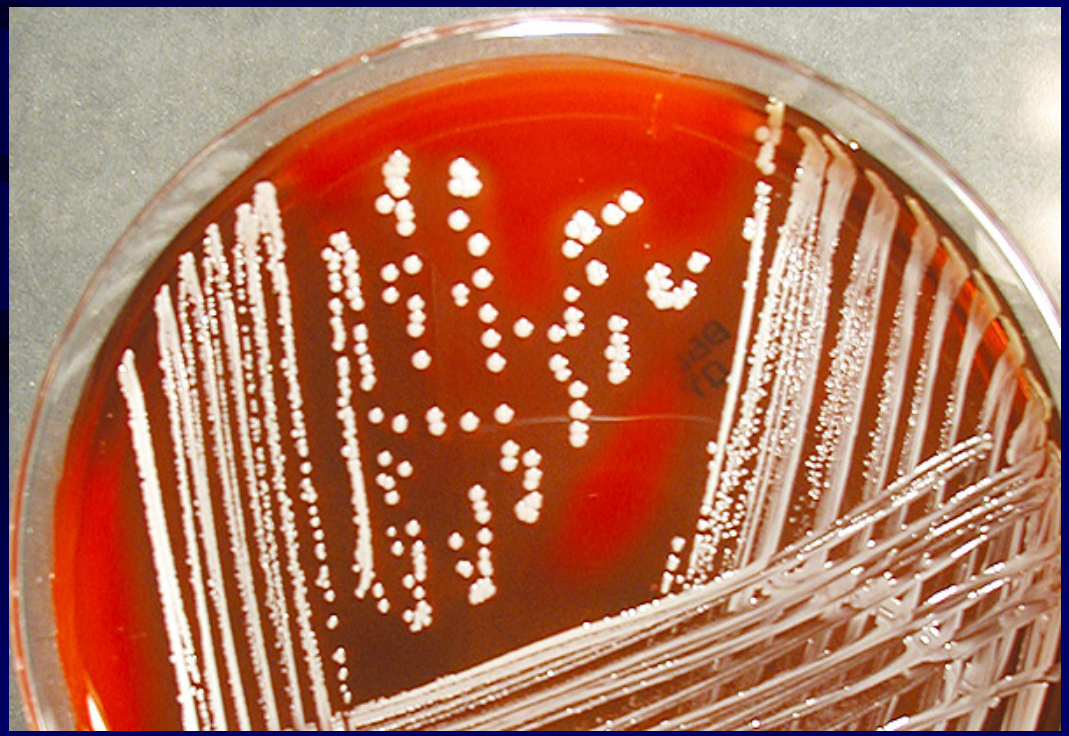
Do PYR if tube coagulase negative to rule out *S. lugdunensis

Case study

- Positive blood culture from patient with endocarditis
- Slide or Latex coagulase positive
- Tube coagulase-negative
- PYR positive
- Ornithine positive

Staphylococcus lugdunensis

- Identification important to treatment
- CLSI now uses S. aureus methicillin breakpoints for this species

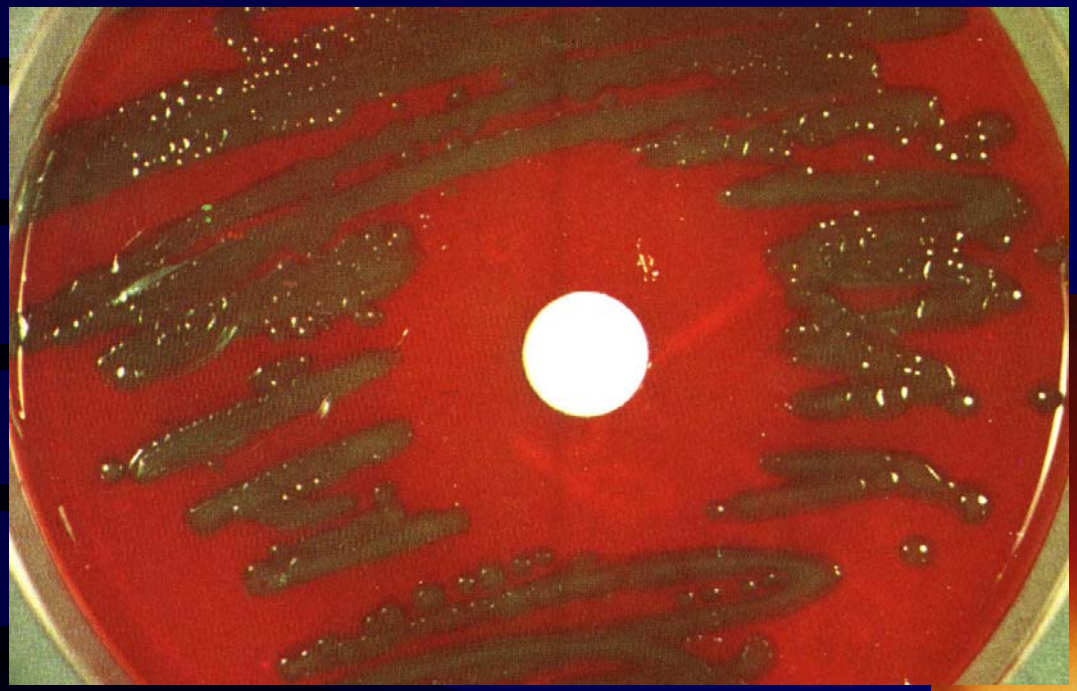


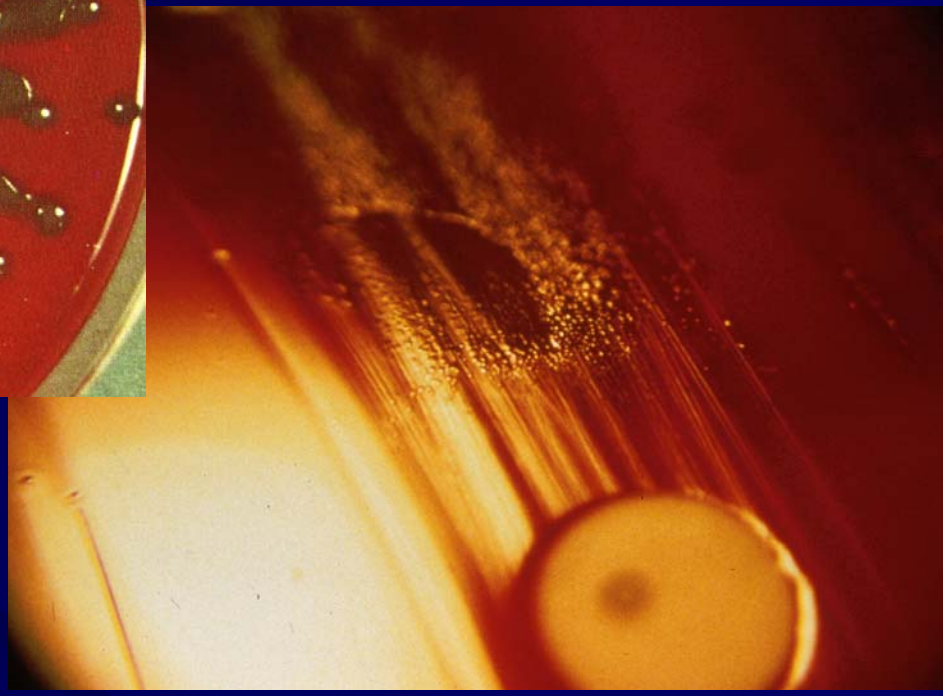
Use PBP2a on CoNS with MICs of less than 4 µg/ml

	Hemolysis 18 h	Slide Coag	Latex agglu	Tube Coag	ODC	Poly B	PYR
S. aureus	+	V	+	+	-	R	-?
S. intermedius (dogs)	V	V	V	+	-	S	+
S. lugdunensis	-	+	+	-	+	R	+
S. schleiferi	-	+	+	-	-	S	+
S. saprophyticus*	-	-	V	-	-	S	-
S. epidermidis	-	-	-	-	V	R	-
most others	-	-	-	-	-	S	V

*Novobiocin resistant.

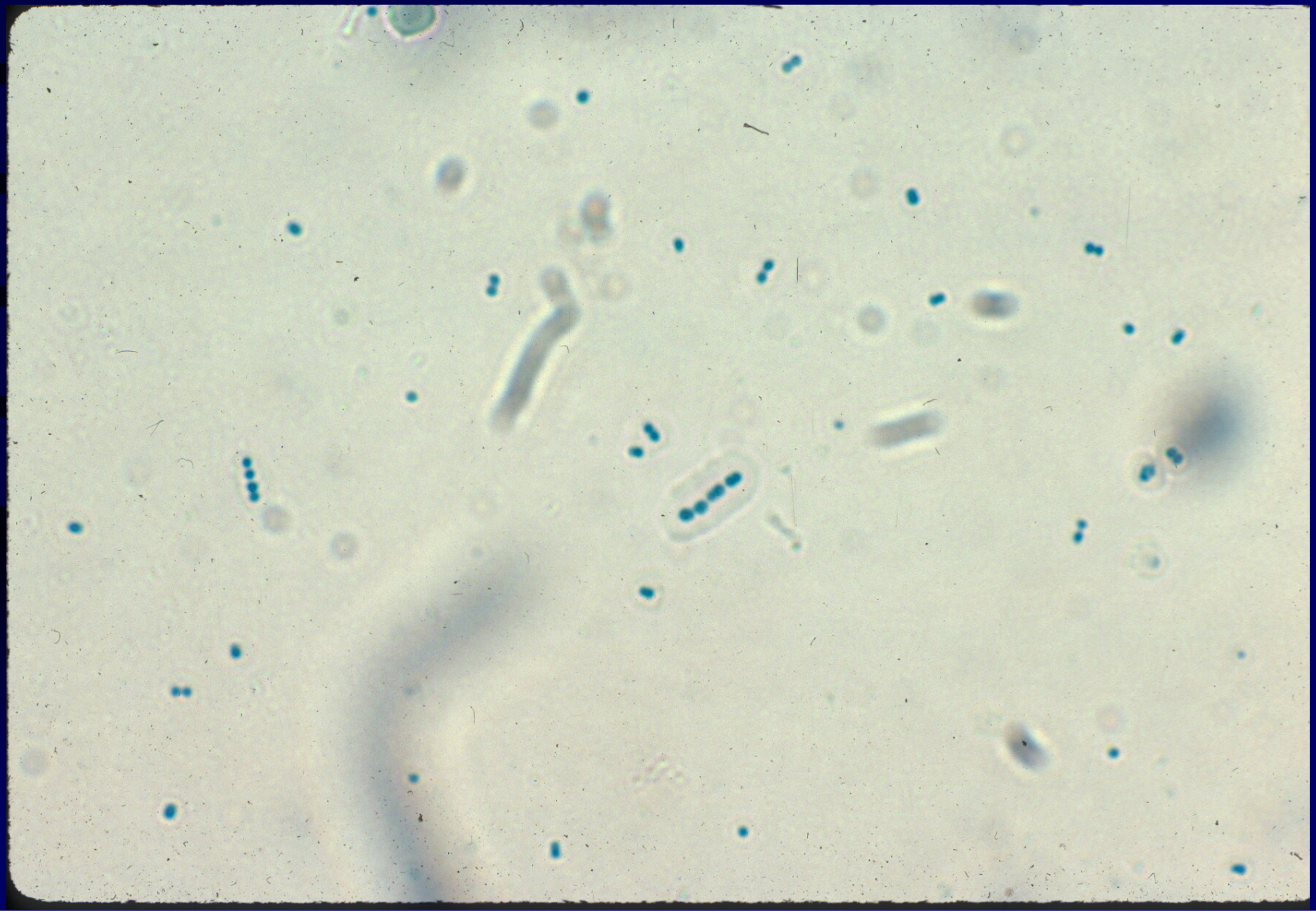
Streptococcus pneumoniae





30 min at 35°C

Quellung reaction



S. pneumoniae Identification

- Colonies small, transparent- may be mucoid
- Gram positive cocci - lancet shaped in pairs
- Catalase negative
- Bile soluble

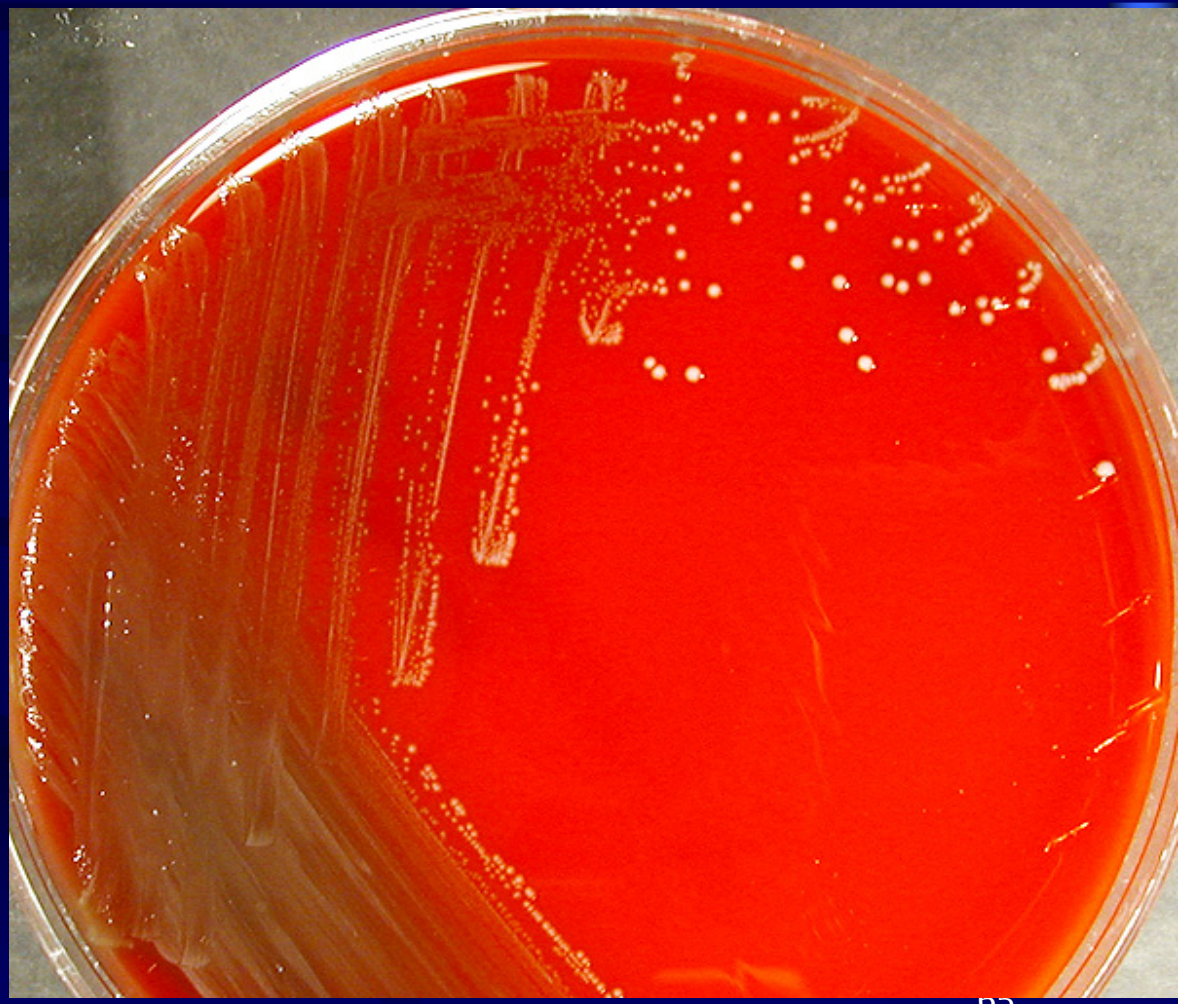
Limitation: Not all are bile soluble but all bile soluble are *S. pneumoniae*

Enterococcus Identification

- Large (1mm) non hemolytic colony
- Gram-positive cocci in pairs and chains
- Catalase-negative
- PYR +

Case Study

- Positive blood culture post-partum
- PYR positive
- Laboratory called it *Enterococcus*
- Gram stain alerted to misidentification
- Called contaminant: patient discharged.



Other PYR-positive Cocci

***Aerococcus* - tetrads**

***Vagococcus* - motile**

Vancomycin resistant is always *Enterococcus* if PYR +

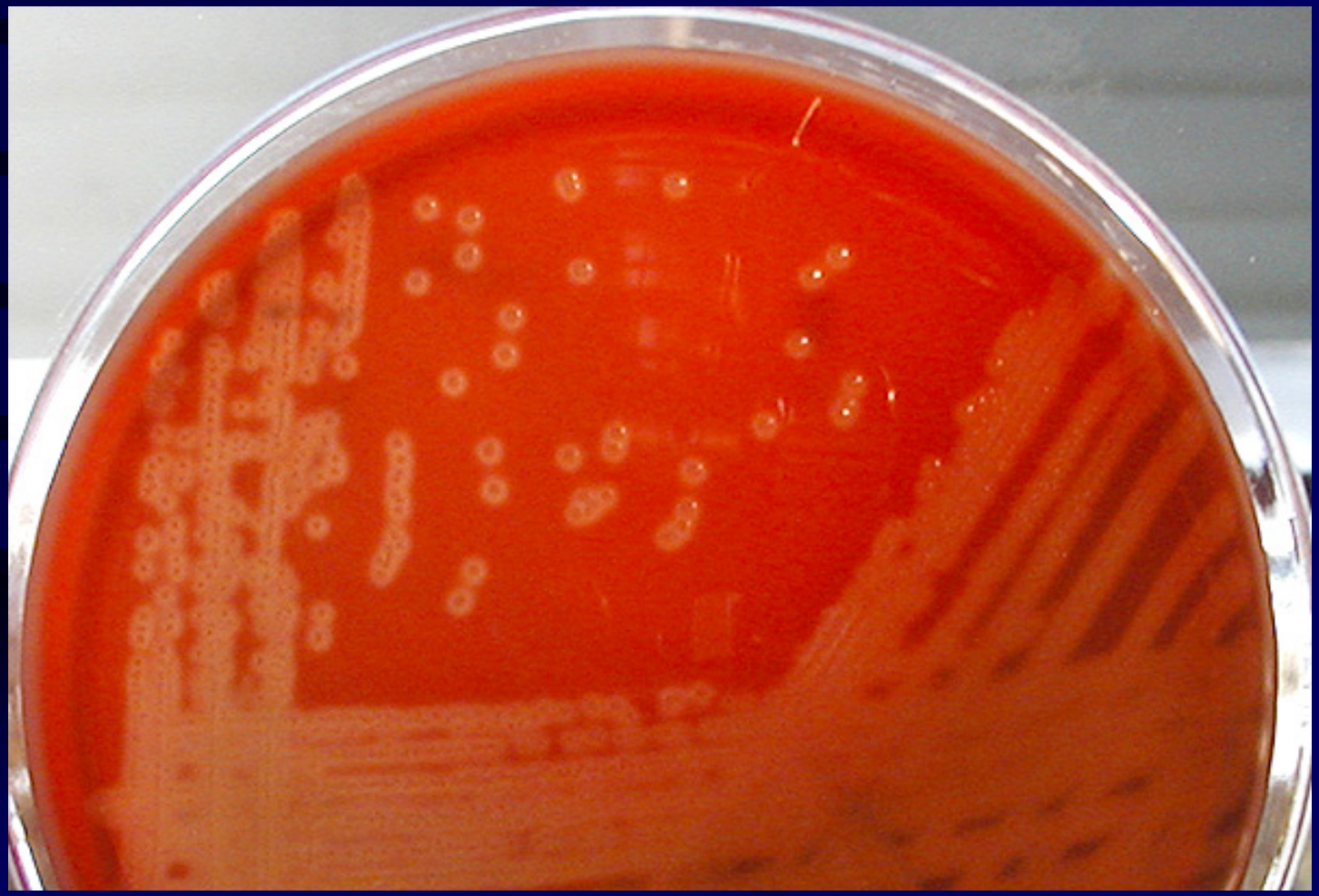
Cannot separate *Enterococcus* from *Lactococcus*

Genus	Phenotypic characteristics[a]							
	Gram	CAT	LAP	NaCl	1O°C	45°C	Colony	Hemolysis on BAP
Enterococccus	ch	–	+	+	+	+	Large	α/γ/β
Lactococcus	ch	–	+	v	+	-	Large	α/γ
Vagococcus (motile)	ch	–	+	+	+	-	Large	α/γ
Abiotrophia/Granulicatella	ch	–	+	-	-	v	Satellite	α/γ
Globicatella	ch	–	-	+	-	-	Small	α
Dolosicoccus	ch	–	-	-	-	-	Small	α
Aerococcus virdans	cl/t	–,w	-		-	-	Large	
Helcococcus kunzii	cl/t	–	-	V	-	-	Small	γ
Gemella	cl/t/ch	–	v	-	-	-	Tiny, 48 h to grow	α/γ
Facklamia	cl/ch	–	+	+	-	-	Small	γ
Alloiococcus	cl/t	w,+	+	+	-	-	Tiny, 72 h to grow	γ
Ignavigranum	cl/ch	–	+	+	-	-	Satellite (v) or small	γ
Rothia mucilaginosa[b]	cl	–,w,+	+	-			Sticky	γ
Dolosigranulum	cl/t	–	+	+	-	-	Small	γ

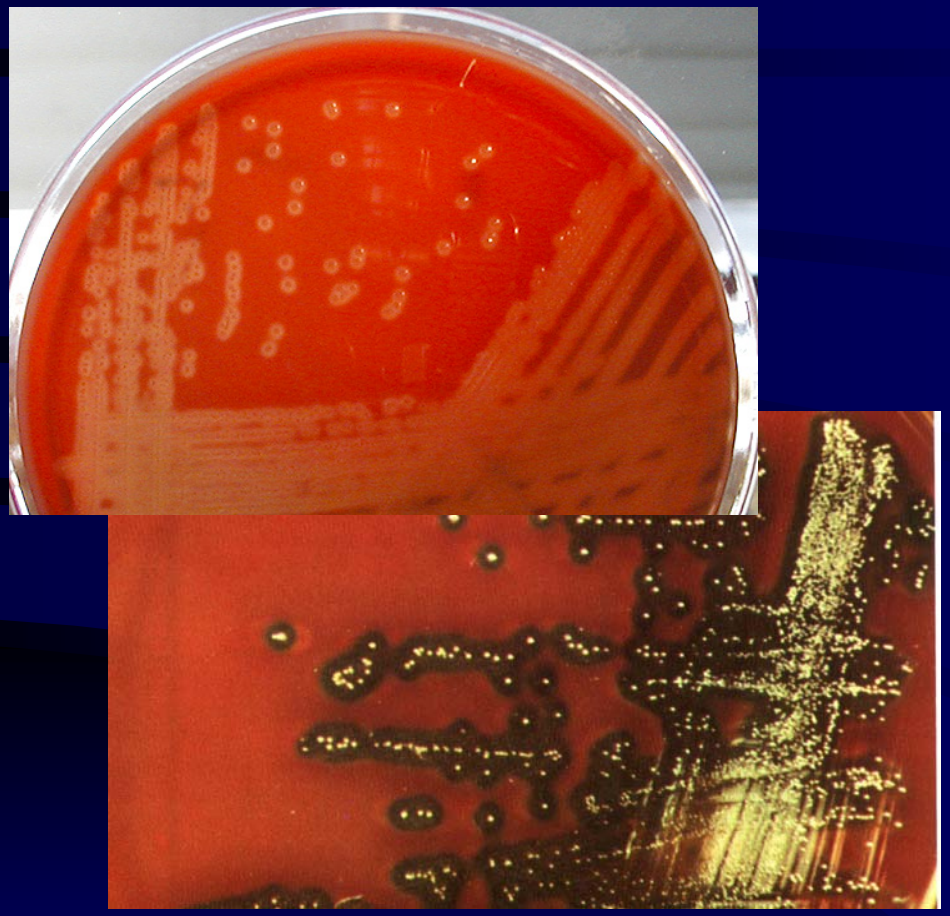
Identification of *S. pyogenes*

- Colonies: dry, peaked >0.5 mm in diameter
- Beta hemolytic
- Catalase-negative
- Gram-positive cocci in pairs and chains
- PYR-positive or positive particle agglutination

Enterococcus can be beta



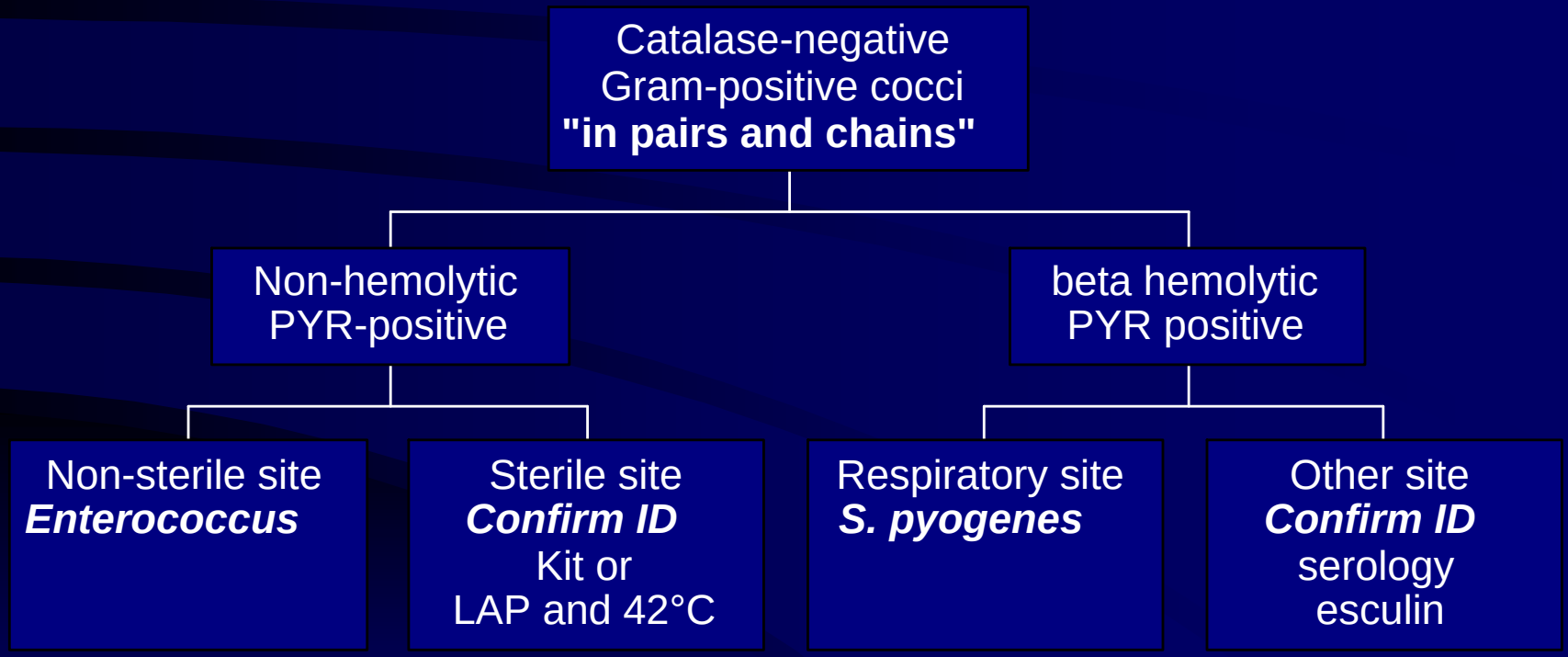
Enterococcus look different



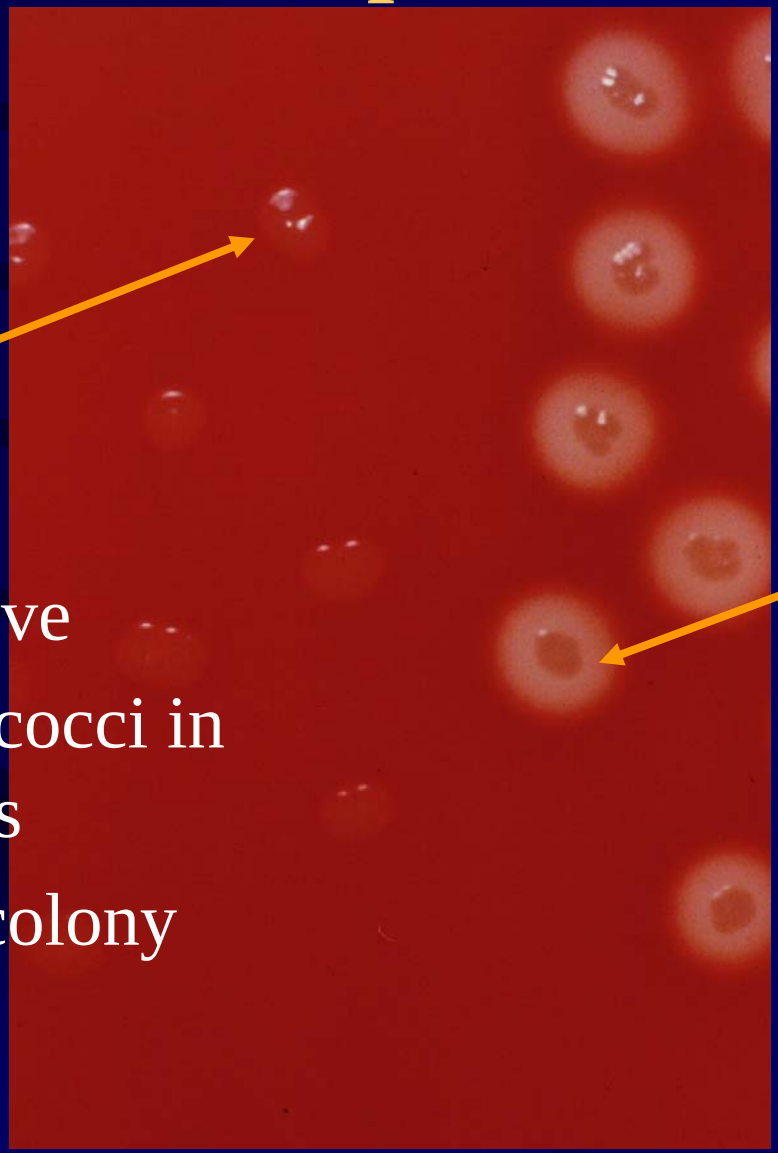
Group A Strep

- *Enterococcus* colonies are larger with less defined ß zone
- Do rapid esculin if there is a concern
- *Enterococci* are esculin-positive

PYR - Positive Algorithm



Beta Streptococci



Group B

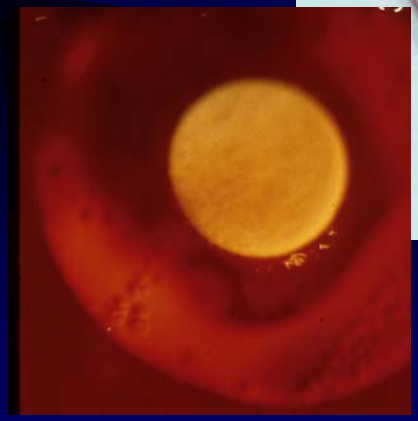
Group G

- Catalase-negative
- Gram-positive cocci in pairs and chains
- Characteristic colony

and CAMP test positive

CAMP +

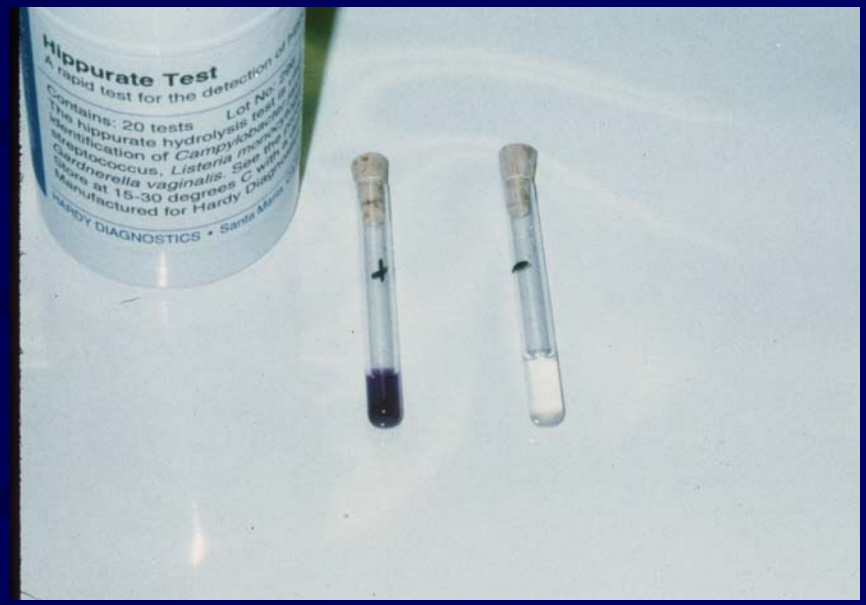
Reverse
CAMP +



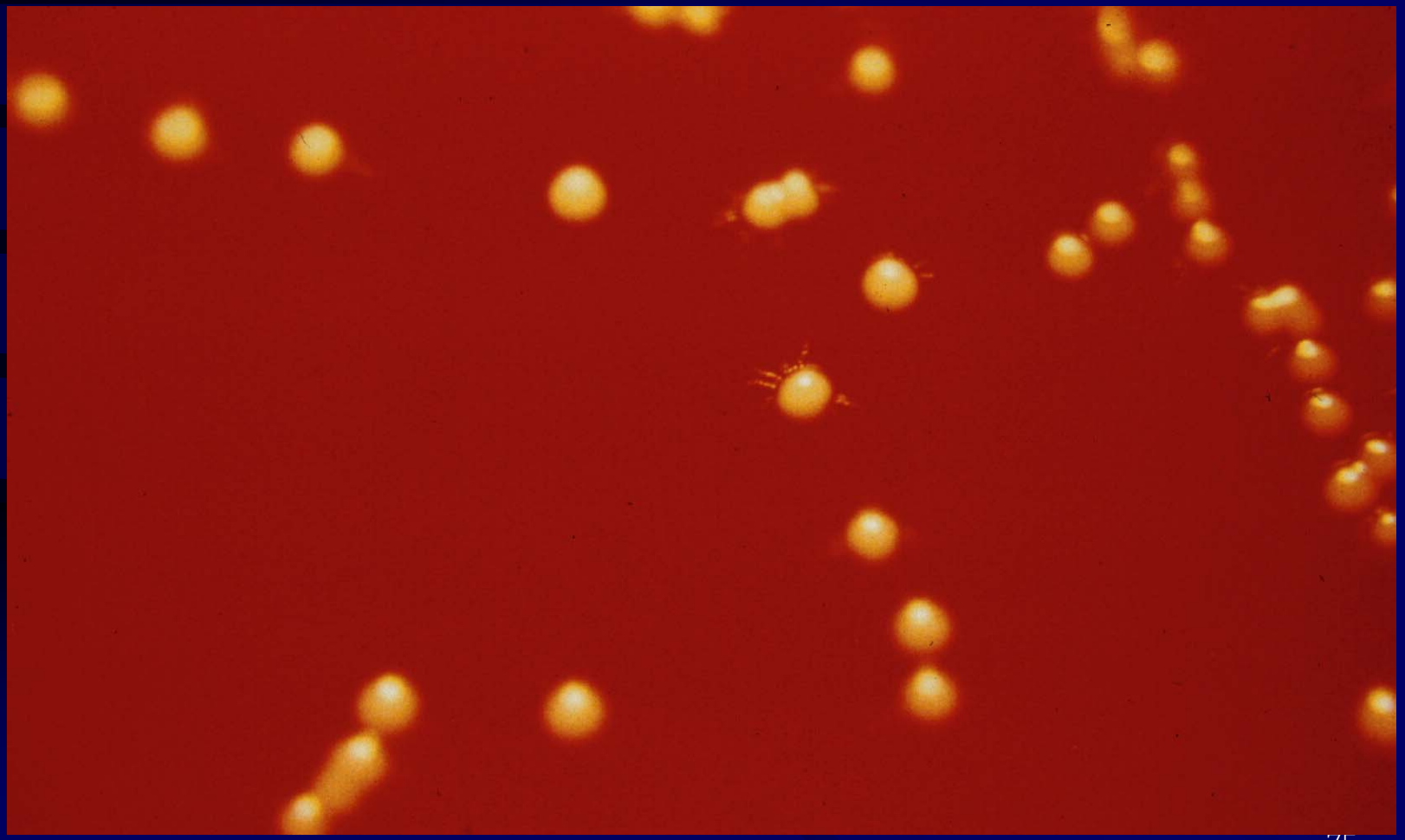
Rapid CAMP-20 minutes at 35°C

...or hippurate positive

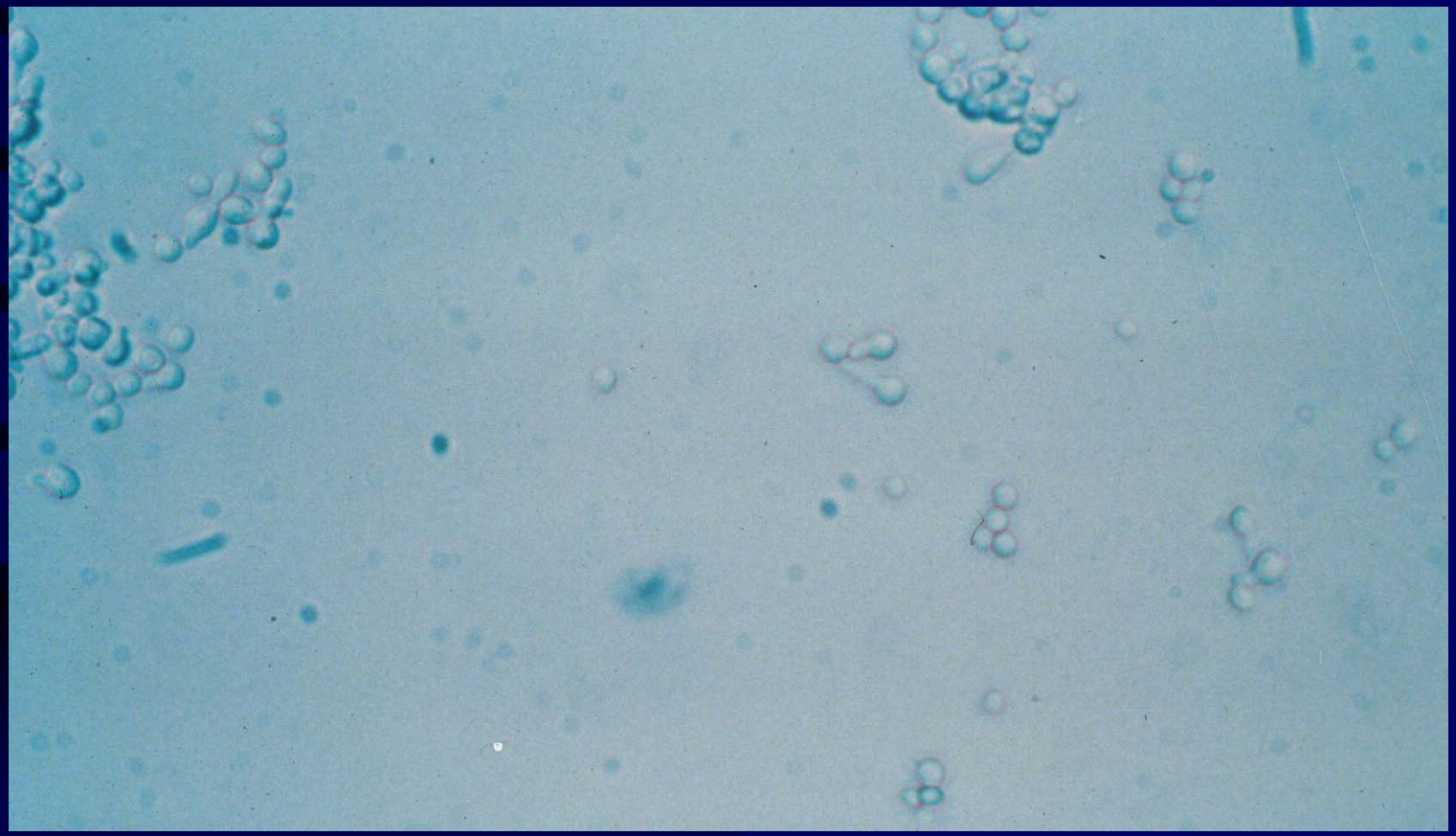
Limitation: Do not do hippurate on non-hemolytic colonies- Other Streptococcus can be positive



Candida albicans



Candida albicans



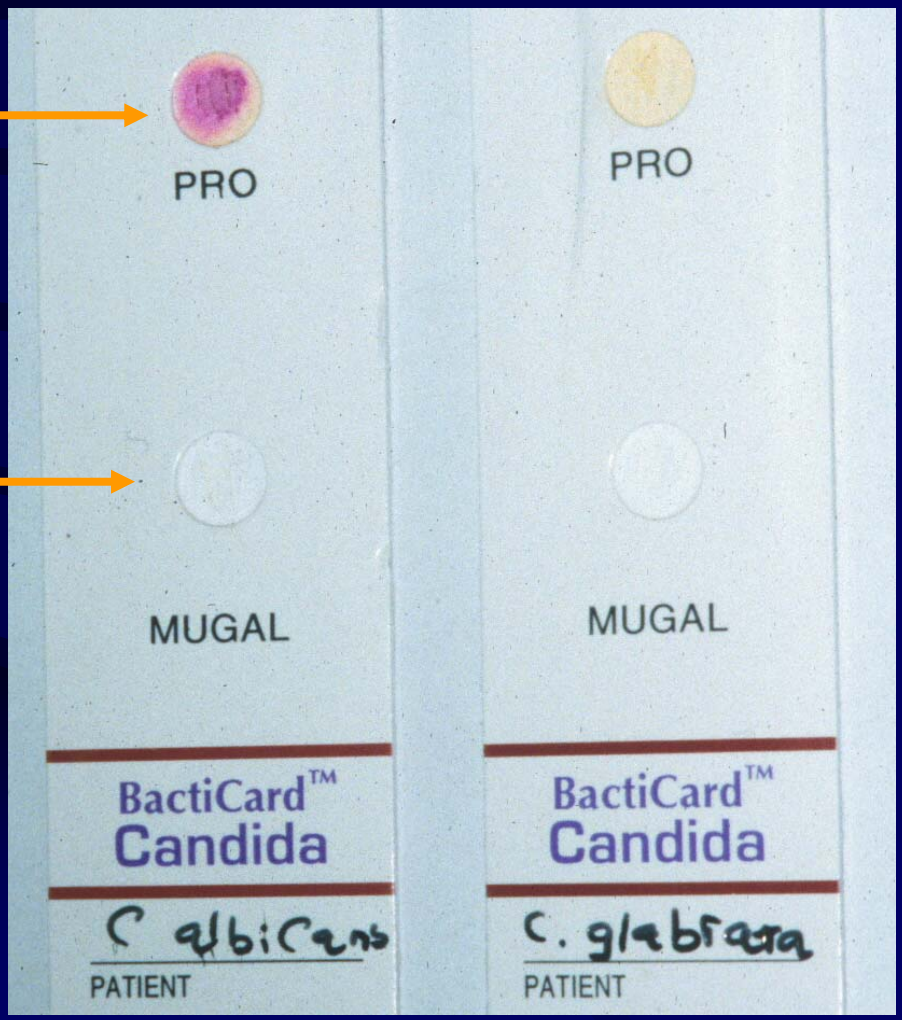
Only use 100% fetal or newborn calf serum-2 h 35°C

Candida albicans = Positive for both enzymes

Add developer at 5 min

Read under UV light Like MUG

Use antibiotic free media



Candida albicans Limitations

- Always start with a wet mount to confirm presence of yeast
- Cannot separate from *C. dubliniensis* which does not grow at 42°C-Not usually necessary
- *C. tropicalis* can form fringe (not feet) or get projections in germ tube after 3 h.

Candida glabrata

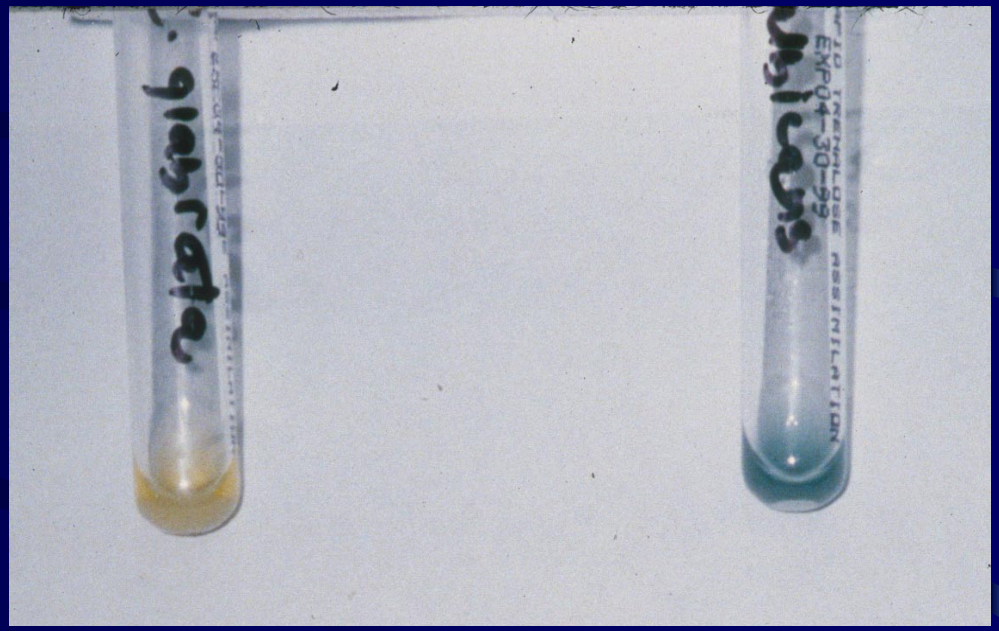
- Colonies smaller on BAP than other yeast
- Yeast in wet mount are tiny with no hyphae
- Colonies are larger on EMB than BAP at 24 h or….

Represents 20% of yeast in urine

Growth in 24 h at 35°C

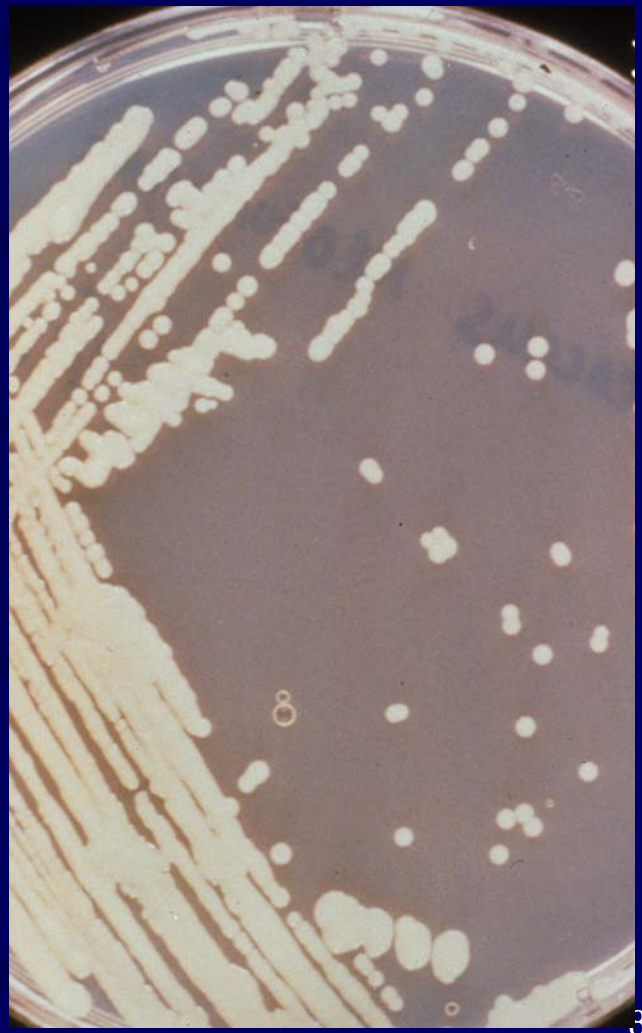
Candida glabrata

- Colonies smaller on BAP than other yeast
- Yeast in wet mount are tiny
- Positive (yellow) RAT test in 3 h at 42°C



Cryptococcus neoformans

- Large mucoid colonies
- No pigment
- Capsule by India Ink or no pseudohyphae
- Round cells
- And positive caffeic acid test



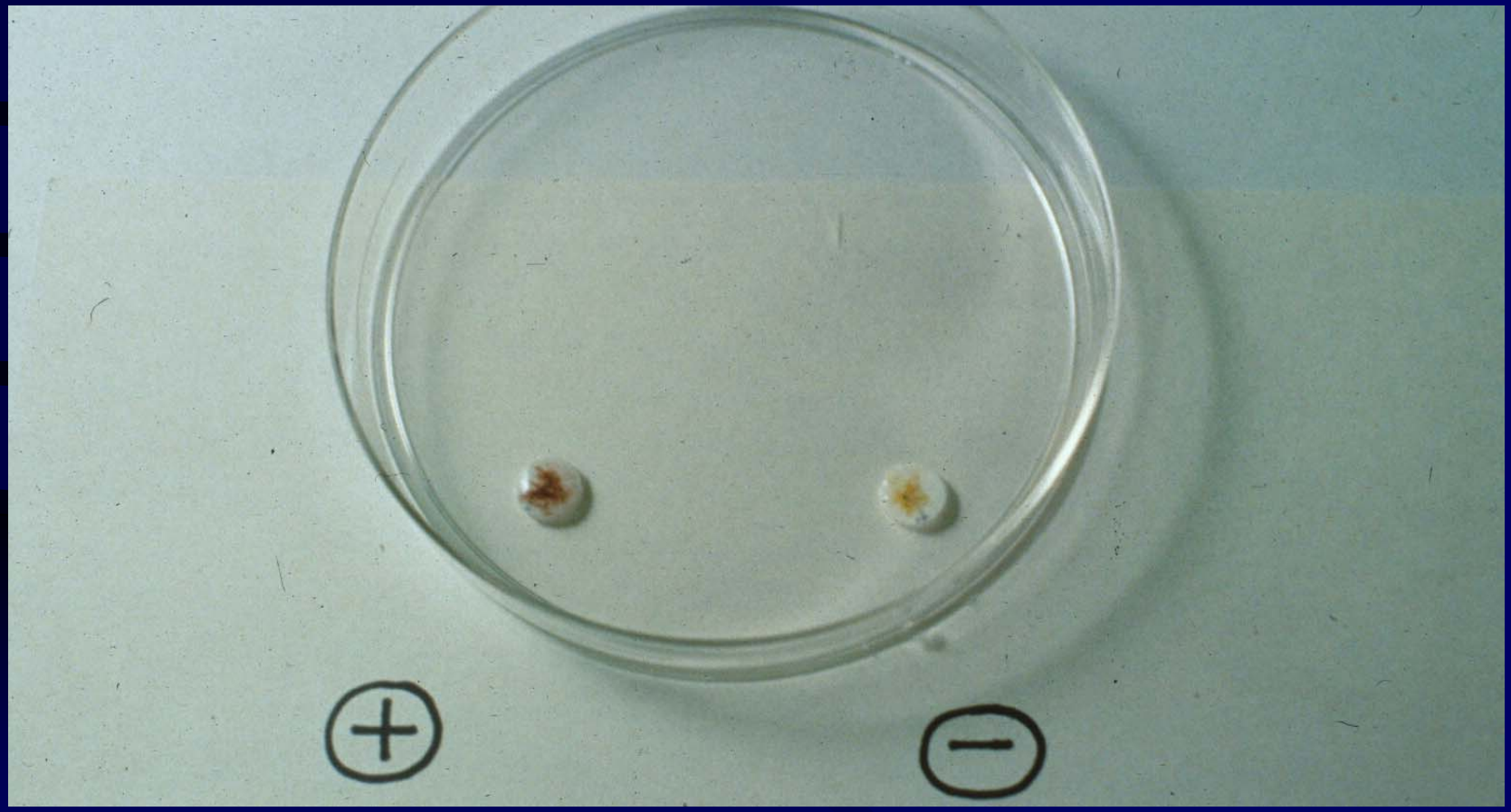
Cryptococcus neoformans

Overnight

Rapid urea

- Rapid caffeic acid
- Use media without dextrose
- 30°C 4 h is best

L-DOPA disk works better



Rapid testing can make a difference

Thank you